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(54) Title: PRODUCTION OF GAMMA LINOLENIC ACID BY A Δ 6-DESATURASE

(57) Abstract

Linoleic acid is converted into γ -linolenic acid by the enzyme Δ 6-desaturase. The present invention is directed to isolated nucleic acids comprising the Δ 6-desaturase gene. More particularly, the isolated nucleic acid comprises the promoter, coding region and termination regions of the Δ 6-desaturase gene. The present invention provides recombinant constructions comprising the Δ 6-desaturase coding region in functional combination with heterologous regulatory sequences. The nucleic acids and recombinant constructions of the instant invention are useful in the production of GLA in transgenic organisms.

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1 PRODUCTION OF GAMMA LINOLENIC ACID BY A Δ6-DESATURASE

Linoleic acid (18:2) (LA) is transformed into gamma linolenic acid (18:3) (GLA) by the enzyme 5 Δ6-desaturase. When this enzyme, or the nucleic acid encoding it, is transferred into LA-producing cells, GLA is produced. The present invention provides nucleic acids comprising the Δ6-desaturase gene. More specifically, the nucleic acids comprise the 10 promoters, coding regions and termination regions of the Δ6-desaturase genes. The present invention is further directed to recombinant constructions comprising a Δ6-desaturase coding region in functional combination with heterologous regulatory sequences. 15 The nucleic acids and recombinant constructions of the instant invention are useful in the production of GLA in transgenic organisms.

Unsaturated fatty acids such as linoleic (C₁₈Δ^{9,12}) and α-linolenic (C₁₈Δ^{9,12,15}) acids are essential 20 dietary constituents that cannot be synthesized by vertebrates since vertebrate cells can introduce double bonds at the Δ⁹ position of fatty acids but cannot introduce additional double bonds between the Δ⁹ double bond and the methyl-terminus of the fatty 25 acid chain. Because they are precursors of other products, linoleic and α-linolenic acids are essential fatty acids, and are usually obtained from plant sources. Linoleic acid can be converted by mammals into γ-linolenic acid (GLA, C₁₈Δ^{6,9,12}) which can in turn 30 be converted to arachidonic acid (20:4), a critically

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1 important fatty acid since it is an essential
precursor of most prostaglandins.

The dietary provision of linoleic acid, by
virtue of its resulting conversion to GLA and
5 arachidonic acid, satisfies the dietary need for GLA
and arachidonic acid. However, a relationship has
been demonstrated between consumption of saturated
fats and health risks such as hypercholesterolemia,
atherosclerosis and other clinical disorders which
10 correlate with susceptibility to coronary disease,
while the consumption of unsaturated fats has been
associated with decreased blood cholesterol
concentration and reduced risk of atherosclerosis.
The therapeutic benefits of dietary GLA may result
15 from GLA being a precursor to arachidonic acid and
thus subsequently contributing to prostaglandin
synthesis. Accordingly, consumption of the more
unsaturated GLA, rather than linoleic acid, has
potential health benefits. However, GLA is not
20 present in virtually any commercially grown crop
plant.

Linoleic acid is converted into GLA by the
enzyme $\Delta 6$ -desaturase. $\Delta 6$ -desaturase, an enzyme of
more than 350 amino acids, has a membrane-bound domain
25 and an active site for desaturation of fatty acids.
When this enzyme is transferred into cells which
endogenously produce linoleic acid but not GLA, GLA is
produced. The present invention, by providing the
gene encoding $\Delta 6$ -desaturase, allows the production of
30 transgenic organisms which contain functional $\Delta 6$ -
desaturase and which produce GLA. In addition to

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1 allowing production of large amounts of GLA, the present invention provides new dietary sources of GLA.

The present invention is directed to isolated $\Delta 6$ -desaturase genes. Specifically, the 5 isolated genes comprises the $\Delta 6$ -desaturase promoters, coding regions, and termination regions.

The present invention is further directed to expression vectors comprising the $\Delta 6$ -desaturase promoter, coding region and termination region.

10 Yet another aspect of this invention is directed to expression vectors comprising a $\Delta 6$ -desaturase coding region in functional combination with heterologous regulatory regions, i.e. elements not derived from the $\Delta 6$ -desaturase gene.

15 Cells and organisms comprising the vectors of the present invention, and progeny of such organisms, are also provided by the present invention.

A further aspect of the present invention provides isolated bacterial $\Delta 6$ -desaturase. An 20 isolated plant $\Delta 6$ -desaturase is also provided.

Yet another aspect of this invention provides a method for producing plants with increased gamma linolenic acid content.

A method for producing chilling tolerant 25 plants is also provided by the present invention.

Fig. 1 depicts the hydropathy profiles of the deduced amino acid sequences of Synechocystis $\Delta 6$ -desaturase (Panel A) and $\Delta 12$ -desaturase (Panel B).

Putative membrane spanning regions are indicated by 30 solid bars. Hydrophobic index was calculated for a

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- 1 window size of 19 amino acid residues [Kyte, et al.
(1982) J. Molec. Biol. 157].

Fig. 2 provides gas liquid chromatography profiles of wild type (Panel A) and transgenic (Panel
5 B) Anabaena.

Fig. 3 is a diagram of maps of cosmid cSy75, cSy13 and Csy7 with overlapping regions and subclones. The origins of subclones of Csy75, Csy75-3.5 and Csy7 are indicated by the dashed diagonal lines.

10 Restriction sites that have been inactivated are in parentheses.

Fig. 4 provides gas liquid chromatography profiles of wild type (Panel A) and transgenic (Panel B) tobacco.

15 Fig. 5A depicts the DNA sequence of a Δ -6 desaturase cDNA isolated from borage.

Fig. 5B depicts the protein sequence of the open reading frame in the isolated borage Δ -6 desaturase cDNA. Three amino acid motifs
20 characteristic of desaturases are indicated and are, in order, lipid box, metal box 1, and metal box 2.

Fig. 6 is a dendrogram showing similarity of the borage Δ 6-desaturase to other membrane-bound desaturases. The amino acid sequence of the borage
25 Δ 6-desaturase was compared to other known desaturases using Gene Works (IntelliGenetics). Numerical values correlate to relative phylogenetic distances between subgroups compared.

Fig. 7 is a restriction map of 221. Δ 6.NOS and 121. Δ 6.NOS. In 221. Δ 6.NOS, the remaining portion
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1 of the plasmid is pBI221 and in 121. Δ 6.NOS, the
remaining portion of the plasmid is pBI121.

Fig. 8 provides gas liquid chromatography profiles of mock transfected (Panel A) and 221. Δ 6.NOS 5 transfected (Panel B) carrot cells. The positions of 18:2, 18:3 α , and 18:3 γ (GLA) are indicated.

Fig. 9 provides gas liquid chromatography profiles of an untransformed tobacco leaf (Panel A) and a tobacco leaf transformed with 121. Δ 6.NOS. The 10 positions of 18:2, 18:3 α , 18:3 γ (GLA), and 18:4 are indicated.

Fig. 10 provides gas liquid chromatography profiles for untransformed tobacco seeds (Panel A) and seeds of tobacco transformed with 121. Δ 6.NOS. The 15 positions of 18:2, 18:3 α and 18:3 γ (GLA) are indicated.

The present invention provides isolated nucleic acids encoding Δ 6-desaturase. To identify a nucleic acid encoding Δ 6-desaturase, DNA is isolated from an organism which produces GLA. Said organism 20 can be, for example, an animal cell, certain fungi (e.g. Mortierella), certain bacteria (e.g. Synechocystis) or certain plants (borage, Oenothera, currants). The isolation of genomic DNA can be accomplished by a variety of methods well-known to one 25 of ordinary skill in the art, as exemplified by Sambrook et al. (1989) in Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, NY. The isolated DNA is fragmented by physical methods or enzymatic digestion and cloned into an appropriate 30 vector, e.g. a bacteriophage or cosmid vector, by any of a variety of well-known methods which can be found

1 in references such as Sambrook *et al.* (1989). Expression vectors containing the DNA of the present invention are specifically contemplated herein. DNA encoding $\Delta 6$ -desaturase can be identified by gain of
5 function analysis. The vector containing fragmented DNA is transferred, for example by infection, transconjugation, transfection, into a host organism that produces linoleic acid but not GLA. As used herein, "transformation" refers generally to the
10 incorporation of foreign DNA into a host cell. Methods for introducing recombinant DNA into a host organism are known to one of ordinary skill in the art and can be found, for example, in Sambrook *et al.* (1989). Production of GLA by these organisms (i.e.,
15 gain of function) is assayed, for example by gas chromatography or other methods known to the ordinarily skilled artisan. Organisms which are induced to produce GLA, i.e. have gained function by the introduction of the vector, are identified as
20 expressing DNA encoding $\Delta 6$ -desaturase, and said DNA is recovered from the organisms. The recovered DNA can again be fragmented, cloned with expression vectors, and functionally assessed by the above procedures to define with more particularity the DNA encoding $\Delta 6$ -
25 desaturase.

As an example of the present invention, random DNA is isolated from the cyanobacteria Synechocystis Pasteur Culture Collection (PCC) 6803, American Type Culture Collection (ATCC) 27184, cloned
30 into a cosmid vector, and introduced by transconjugation into the GLA-deficient cyanobacterium

1 1 Anabaena strain PCC 7120, ATCC 27893. Production of GLA from Anabaena linoleic acid is monitored by gas chromatography and the corresponding DNA fragment is isolated.

5 5 The isolated DNA is sequenced by methods well-known to one of ordinary skill in the art as found, for example, in Sambrook et al. (1989).

In accordance with the present invention, DNA molecules comprising $\Delta 6$ -desaturase genes have been 10 isolated. More particularly, a 3.588 kilobase (kb) DNA comprising a $\Delta 6$ -desaturase gene has been isolated from the cyanobacteria Synechocystis. The nucleotide sequence of the 3.588 kb DNA was determined and is shown in SEQ ID NO:1. Open reading frames defining 15 potential coding regions are present from nucleotide 317 to 1507 and from nucleotide 2002 to 3081. To define the nucleotides responsible for encoding $\Delta 6$ -desaturase, the 3.588 kb fragment that confers $\Delta 6$ -desaturase activity is cleaved into two subfragments, 20 each of which contains only one open reading frame. Fragment ORF1 contains nucleotides 1 through 1704, while fragment ORF2 contains nucleotides 1705 through 3588. Each fragment is subcloned in both forward and reverse orientations into a conjugal expression vector 25 (AM542, Wolk et al. [1984] Proc. Natl. Acad. Sci. USA 81, 1561) that contains a cyanobacterial carboxylase promoter. The resulting constructs (i.e. ORF1(F), ORF1(R), ORF2(F) and ORF2(R)] are conjugated to wild-type Anabaena PCC 7120 by standard methods (see, for 30 example, Wolk et al. (1984) Proc. Natl. Acad. Sci. USA 81, 1561). Conjugated cells of Anabaena are

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1 identified as Neo^r green colonies on a brown
background of dying non-conjugated cells after two
weeks of growth on selective media (standard mineral
media BG11N + containing 30 μ g/ml of neomycin according
5 to Rippka et al., (1979) J. Gen Microbiol. 111, 1).
The green colonies are selected and grown in selective
liquid media (BG11N + with 15 μ g/ml neomycin). Lipids
are extracted by standard methods (e.g. Dahmer et al.,
(1989) Journal of American Oil Chemical Society 66,
10 543) from the resulting transconjugants containing the
forward and reverse oriented ORF1 and ORF2 constructs.
For comparison, lipids are also extracted from wild-
type cultures of Anabaena and Synechocystis. The
fatty acid methyl esters are analyzed by gas liquid
15 chromatography (GLC), for example with a Tracor-560
gas liquid chromatograph equipped with a hydrogen
flame ionization detector and a capillary column. The
results of GLC analysis are shown in Table 1.

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1 Table 1: Occurrence of C18 fatty acids in wild-type
 and transgenic cyanobacteria

	SOURCE	18:0	18:1	18:2	γ 18:3	α 18:3	18:4
5	Anabaena (wild type)	+	+	+	-	+	-
	Anabaena + ORF1 (F)	+	+	+	-	+	-
	Anabaena + ORF1 (R)	+	+	+	-	+	-
10	Anabaena + ORF2 (F)	+	+	+	+	+	+
	Anabaena + ORF2 (R)	+	+	+	-	+	-
	Synechocystis (wild type)	+	+	+	+	-	-

As assessed by GLC analysis, GLA deficient
 15 Anabaena gain the function of GLA production when the
 construct containing ORF2 in forward orientation is
 introduced by transconjugation. Transconjugants
 containing constructs with ORF2 in reverse orientation
 to the carboxylase promoter, or ORF1 in either
 20 orientation, show no GLA production. This analysis
 demonstrates that the single open reading frame (ORF2)
 within the 1884 bp fragment encodes $\Delta 6$ -desaturase.
 The 1884 bp fragment is shown as SEQ ID NO:3. This is
 substantiated by the overall similarity of the
 25 hydrophathy profiles between $\Delta 6$ -desaturase and $\Delta 12$ -
 desaturase [Wada et al. (1990) Nature 347] as shown in
 Fig. 1 as (A) and (B), respectively.

Also in accordance with the present
 invention, a cDNA comprising a $\Delta 6$ -desaturase gene from
 30 borage (Borago officinalis) has been isolated. The
 nucleotide sequence of the 1.685 kilobase (kb) cDNA

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1 was determined and is shown in Fig. 5A (SEQ ID NO: 4).
The ATG start codon and stop codon are underlined.
The amino acid sequence corresponding to the open
reading frame in the borage delta 6-desaturase is
5 shown in Fig. 5B (SEQ ID NO: 5).

Isolated nucleic acids encoding Δ 6-
desaturase can be identified from other GLA-producing
organisms by the gain of function analysis described
above, or by nucleic acid hybridization techniques
10 using the isolated nucleic acid which encodes
Synechocystis or borage Δ 6-desaturase as a
hybridization probe. Both genomic and cDNA cloning
methods are known to the skilled artisan and are
contemplated by the present invention. The
15 hybridization probe can comprise the entire DNA
sequence disclosed as SEQ. ID NO:1 or SEQ. ID NO:4, or
a restriction fragment or other DNA fragment thereof,
including an oligonucleotide probe. Methods for
cloning homologous genes by cross-hybridization are
20 known to the ordinarily skilled artisan and can be
found, for example, in Sambrook (1989) and Beltz et
al. (1983) Methods in Enzymology 100, 266.

In another method of identifying a delta 6-
desaturase gene from an organism producing GLA, a cDNA
25 library is made from poly-A' RNA isolated from
polysomal RNA. In order to eliminate hyper-abundant
expressed genes from the cDNA population, cDNAs or
fragments thereof corresponding to hyper-abundant
cDNAs genes are used as hybridization probes to the
30 cDNA library. Non hybridizing plaques are excised and
the resulting bacterial colonies are used to inoculate

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1 liquid cultures and sequenced. For example, as a
means of eliminating other seed storage protein cDNAs
from a cDNA library made from borage polysomal RNA,
cDNAs corresponding to abundantly expressed seed
5 storage proteins are first hybridized to the cDNA
library. The "subtracted" DNA library is then used to
generate expressed sequence tags (ESTs) and such tags
are used to scan a data base such as GenBank to
identify potential desaturases.

10 Transgenic organisms which gain the function
of GLA production by introduction of DNA encoding Δ -
desaturase also gain the function of
octadecatetraenoic acid (18:4 6,9,12,15) production.
Octadecatetraenoic acid is present normally in fish
15 oils and in some plant species of the Boraginaceae
family (Craig *et al.* [1964] J. Amer. Oil Chem. Soc.
41, 209-211; Gross *et al.* [1976] Can. J. Plant Sci.
56, 659-664). In the transgenic organisms of the
present invention, octadecatetraenoic acid results
20 from further desaturation of α -linolenic acid by $\Delta 6$ -
desaturase or desaturation of GLA by $\Delta 15$ -desaturase.

The 359 amino acids encoded by ORF2, i.e.
the open reading frame encoding Synechocystis $\Delta 6$ -
desaturase, are shown as SEQ. ID NO:2. The open
25 reading frame encoding the borage $\Delta 6$ -desaturase is
shown in SEQ ID NO: 5. The present invention further
contemplates other nucleotide sequences which encode
the amino acids of SEQ ID NO:2 and SEQ ID NO: 5. It
is within the ken of the ordinarily skilled artisan to
30 identify such sequences which result, for example,
from the degeneracy of the genetic code. Furthermore,

1 one of ordinary skill in the art can determine, by the
gain of function analysis described hereinabove,
smaller subfragments of the fragments containing the
open reading frames which encode $\Delta 6$ -desaturases.

5 The present invention contemplates any such
polypeptide fragment of $\Delta 6$ -desaturase and the nucleic
acids therefor which retain activity for converting LA
to GLA.

In another aspect of the present invention,
10 a vector containing a nucleic acid of the present
invention or a smaller fragment containing the
promoter, coding sequence and termination region of a
 $\Delta 6$ -desaturase gene is transferred into an organism,
for example, cyanobacteria, in which the $\Delta 6$ -desaturase
15 promoter and termination regions are functional.
Accordingly, organisms producing recombinant $\Delta 6$ -
desaturase are provided by this invention. Yet
another aspect of this invention provides isolated $\Delta 6$ -
desaturase, which can be purified from the recombinant
20 organisms by standard methods of protein purification.
(For example, see Ausubel *et al.* [1987] Current
Protocols in Molecular Biology, Green Publishing
Associates, New York).

Vectors containing DNA encoding $\Delta 6$ -
25 desaturase are also provided by the present invention.
It will be apparent to one of ordinary skill in the
art that appropriate vectors can be constructed to
direct the expression of the $\Delta 6$ -desaturase coding
sequence in a variety of organisms. Replicable
30 expression vectors are particularly preferred.
Replicable expression vectors as described herein are

1 DNA or RNA molecules engineered for controlled
expression of a desired gene, i.e. the $\Delta 6$ -desaturase
gene. Preferably the vectors are plasmids,
bacteriophages, cosmids or viruses. Shuttle vectors,
5 e.g. as described by Wolk *et al.* (1984) Proc. Natl.
Acad. Sci. USA, 1561-1565 and Bustos *et al.* (1991) J.
Bacteriol. 174, 7525-7533, are also contemplated in
accordance with the present invention. Sambrook *et*
al. (1989), Goeddel, ed. (1990) Methods in Enzymology
10 185 Academic Press, and Perbal (1988) A Practical
Guide to Molecular Cloning, John Wiley and Sons, Inc.,
provide detailed reviews of vectors into which a
nucleic acid encoding the present $\Delta 6$ -desaturase can be
inserted and expressed. Such vectors also contain
15 nucleic acid sequences which can effect expression of
nucleic acids encoding $\Delta 6$ -desaturase. Sequence
elements capable of effecting expression of a gene
product include promoters, enhancer elements, upstream
activating sequences, transcription termination
20 signals and polyadenylation sites. Both constitutive
and tissue specific promoters are contemplated. For
transformation of plant cells, the cauliflower mosaic
virus (CaMV) 35S promoter and promoters which are
regulated during plant seed maturation are of
25 particular interest. All such promoter and
transcriptional regulatory elements, singly or in
combination, are contemplated for use in the present
replicable expression vectors and are known to one of
ordinary skill in the art. The CaMV 35S promoter is
described, for example, by Restrepo *et al.* (1990)
30

1 1 Plant Cell 2, 987. Genetically engineered and mutated
regulatory sequences are also contemplated.

The ordinarily skilled artisan can determine
vectors and regulatory elements suitable for
5 expression in a particular host cell. For example, a
vector comprising the promoter from the gene encoding
the carboxylase of Anabaena operably linked to the
coding region of $\Delta 6$ -desaturase and further operably
linked to a termination signal from Synechocystis is
10 appropriate for expression of $\Delta 6$ -desaturase in
cyanobacteria. "Operably linked" in this context
means that the promoter and terminator sequences
effectively function to regulate transcription. As a
further example, a vector appropriate for expression
15 of $\Delta 6$ -desaturase in transgenic plants can comprise a
seed-specific promoter sequence derived from
helianthinin, napin, or glycinin operably linked to
the $\Delta 6$ -desaturase coding region and further operably
linked to a seed termination signal or the nopaline
20 synthase termination signal. As a still further
example, a vector for use in expression of $\Delta 6$ -
desaturase in plants can comprise a constitutive
promoter or a tissue specific promoter operably linked
to the $\Delta 6$ -desaturase coding region and further
25 operably linked to a constitutive or tissue specific
terminator or the nopaline synthase termination
signal.

In particular, the helianthinin regulatory
elements disclosed in applicant's copending U.S.
30 Application Serial No. 682,354, filed April 8, 1991
and incorporated herein by reference, are contemplated

1 as promoter elements to direct the expression of the
Δ6-desaturase of the present invention.

5 Modifications of the nucleotide sequences or
regulatory elements disclosed herein which maintain
the functions contemplated herein are within the scope
of this invention. Such modifications include
insertions, substitutions and deletions, and
specifically substitutions which reflect the
degeneracy of the genetic code.

10 Standard techniques for the construction of
such hybrid vectors are well-known to those of
ordinary skill in the art and can be found in
references such as Sambrook *et al.* (1989), or any of
the myriad of laboratory manuals on recombinant DNA
15 technology that are widely available. A variety of
strategies are available for ligating fragments of
DNA, the choice of which depends on the nature of the
termini of the DNA fragments. It is further
contemplated in accordance with the present invention
20 to include in the hybrid vectors other nucleotide
sequence elements which facilitate cloning, expression
or processing, for example sequences encoding signal
peptides, a sequence encoding KDEL, which is required
for retention of proteins in the endoplasmic reticulum
25 or sequences encoding transit peptides which direct
Δ6-desaturase to the chloroplast. Such sequences are
known to one of ordinary skill in the art. An
optimized transit peptide is described, for example,
by Van den Broeck *et al.* (1985) Nature 313, 358.
30 Prokaryotic and eukaryotic signal sequences are

1 disclosed, for example, by Michaelis et al. (1982)
Ann. Rev. Microbiol. 36, 425.

A further aspect of the instant invention provides organisms other than cyanobacteria or plants
5 which contain the DNA encoding the $\Delta 6$ -desaturase of the present invention. The transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, and plants and animals. The isolated DNA of the present invention
10 can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in references such as Sambrook et al. (1989).
15

A variety of plant transformation methods are known. The $\Delta 6$ -desaturase gene can be introduced into plants by a leaf disk transformation-regeneration procedure as described by Horsch et al. (1985) Science
20 227, 1229. Other methods of transformation, such as protoplast culture (Horsch et al. (1984) Science 223, 496; DeBlock et al. (1984) EMBO J. 2, 2143; Barton et al. (1983) Cell 32, 1033) can also be used and are within the scope of this invention. In a preferred
25 embodiment plants are transformed with Agrobacterium-derived vectors. However, other methods are available to insert the $\Delta 6$ -desaturase genes of the present invention into plant cells. Such alternative methods include biostatic approaches (Klein et al. (1987) Nature 327, 70), electroporation, chemically-induced DNA uptake, and use of viruses or pollen as vectors.
30

1 When necessary for the transformation
method, the $\Delta 6$ -desaturase genes of the present
invention can be inserted into a plant transformation
vector, e.g. the binary vector described by Bevan
5 (1984) Nucleic Acids Res. 12, 8111. Plant
transformation vectors can be derived by modifying the
natural gene transfer system of Agrobacterium
tumefaciens. The natural system comprises large Ti
(tumor-inducing)-plasmids containing a large segment,
10 known as T-DNA, which is transferred to transformed
plants. Another segment of the Ti plasmid, the vir
region, is responsible for T-DNA transfer. The T-DNA
region is bordered by terminal repeats. In the
modified binary vectors the tumor-inducing genes have
15 been deleted and the functions of the vir region are
utilized to transfer foreign DNA bordered by the T-DNA
border sequences. The T-region also contains a
selectable marker for antibiotic resistance, and a
multiple cloning site for inserting sequences for
20 transfer. Such engineered strains are known as
"disarmed" A. tumefaciens strains, and allow the
efficient transformation of sequences bordered by the
T-region into the nuclear genomes of plants.

25 Surface-sterilized leaf disks are inoculated
with the "disarmed" foreign DNA-containing A.
tumefaciens, cultured for two days, and then
transferred to antibiotic-containing medium.
Transformed shoots are selected after rooting in
medium containing the appropriate antibiotic,
30 transferred to soil and regenerated.

1 Another aspect of the present invention provides transgenic plants or progeny of these plants containing the isolated DNA of the invention. Both monocotyledenous and dicotyledenous plants are
5 contemplated. Plant cells are transformed with the isolated DNA encoding $\Delta 6$ -desaturase by any of the plant transformation methods described above. The transformed plant cell, usually in a callus culture or leaf disk, is regenerated into a complete transgenic
10 plant by methods well-known to one of ordinary skill in the art (e.g. Horsch *et al.* (1985) Science 227, 1129). In a preferred embodiment, the transgenic plant is sunflower, oil seed rape, maize, tobacco, peanut or soybean. Since progeny of transformed
15 plants inherit the DNA encoding $\Delta 6$ -desaturase, seeds or cuttings from transformed plants are used to maintain the transgenic plant line.

The present invention further provides a method for providing transgenic plants with an increased content of GLA. This method includes introducing DNA encoding $\Delta 6$ -desaturase into plant cells which lack or have low levels of GLA but contain LA, and regenerating plants with increased GLA content from the transgenic cells. In particular,
20 commercially grown crop plants are contemplated as the transgenic organism, including, but not limited to, sunflower, soybean, oil seed rape, maize, peanut and tobacco.

The present invention further provides a method for providing transgenic organisms which contain GLA. This method comprises introducing DNA

1 encoding $\Delta 6$ -desaturase into an organism which lacks or
has low levels of GLA, but contains LA. In another
embodiment, the method comprises introducing one or
more expression vectors which comprise DNA encoding
5 $\Delta 12$ -desaturase and $\Delta 6$ -desaturase into organisms which
are deficient in both GLA and LA. Accordingly,
organisms deficient in both LA and GLA are induced to
produce LA by the expression of $\Delta 12$ -desaturase, and
GLA is then generated due to the expression of $\Delta 6$ -
10 desaturase. Expression vectors comprising DNA
encoding $\Delta 12$ -desaturase, or $\Delta 12$ -desaturase and $\Delta 6$ -
desaturase, can be constructed by methods of
recombinant technology known to one of ordinary skill
in the art (Sambrook *et al.*, 1989) and the published
15 sequence of $\Delta 12$ -desaturase (Wada *et al* [1990] Nature
(London) 347, 200-203. In addition, it has been
discovered in accordance with the present invention
that nucleotides 2002-3081 of SEQ. ID NO:1 encode
cyanobacterial $\Delta 12$ -desaturase. Accordingly, this
20 sequence can be used to construct the subject
expression vectors. In particular, commercially grown
crop plants are contemplated as the transgenic
organism, including, but not limited to, sunflower,
soybean, oil seed rape, maize, peanut and tobacco.

25 The present invention is further directed to
a method of inducing chilling tolerance in plants.
Chilling sensitivity may be due to phase transition of
lipids in cell membranes. Phase transition
temperature depends upon the degree of unsaturation of
30 fatty acids in membrane lipids, and thus increasing
the degree of unsaturation, for example by introducing

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1 $\Delta 6$ -desaturase to convert LA to GLA, can induce or
improve chilling resistance. Accordingly, the present
method comprises introducing DNA encoding $\Delta 6$ -
desaturase into a plant cell, and regenerating a plant
5 with improved chilling resistance from said
transformed plant cell. In a preferred embodiment,
the plant is a sunflower, soybean, oil seed rape,
maize, peanut or tobacco plant.

The following examples further illustrate
10 the present invention.

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EXAMPLE 1
Strains and Culture Conditions

- Synechocystis (PCC 6803, ATCC 27184),
5 Anabaena (PCC 7120, ATCC 27893) and Synechococcus (PCC
7942, ATCC 33912) were grown photoautotrophically at
30°C in BG11N+ medium (Rippka *et al.* [1979] J. Gen.
Microbiol. 111, 1-61) under illumination of
incandescent lamps
10 (60μE.m⁻².S⁻¹). Cosmids and plasmids were selected and
propagated in Escherichia coli strain DH5α on LB
medium supplemented with antibiotics at standard
concentrations as described by Maniatis *et al.* (1982)
Molecular Cloning: A Laboratory Manual, Cold Spring
15 Harbor Laboratory, Cold Spring, New York.

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EXAMPLE 2

Construction of Synechocystis Cosmid Genomic Library

Total genomic DNA from Synechocystis (PCC
5 6803) was partially digested with Sau3A and
fractionated on a sucrose gradient (Ausubel et al..
[1987] Current Protocols in Molecular Biology, Greene
Publishing Associates and Wiley Interscience, New
York). Fractions containing 30 to 40 kb DNA fragments
10 were selected and ligated into the dephosphorylated
BamHI site of the cosmid vector, pDUC7 (Buikema et
al. [1991] J. Bacteriol. 173, 1879-1885). The ligated
DNA was packaged in vitro as described by Ausubel et
al. (1987), and packaged phage were propagated in E.
15 coli DH5 α containing the AvaI and Eco4711 methylase
helper plasmid, pRL528 as described by Buikema et al..
(1991). A total of 1152 colonies were isolated
randomly and maintained individually in twelve 96-well
microtiter plates.

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EXAMPLE 3

Gain-of-Function Expression of GLA in Anabaena

5 Anabaena (PCC 7120), a filamentous cyanobacterium, is deficient in GLA but contains significant amounts of linoleic acid, the precursor for GLA (Figure 2; Table 2). The Synechocystis cosmid library described in Example 2 was conjugated into Anabaena (PCC 7120) to identify transconjugants that

10 produce GLA. Anabaena cells were grown to mid-log phase in BG11N+ liquid medium and resuspended in the same medium to a final concentration of approximately 2×10^8 cells per ml. A mid-log phase culture of E. coli RP4 (Burkardt *et al.* [1979] J. Gen. Microbiol.

15 114, 341-348) grown in LB containing ampicillin was washed and resuspended in fresh LB medium. Anabaena and RP4 were then mixed and spread evenly on BG11N+ plates containing 5% LB. The cosmid genomic library was replica plated onto LB plates containing 50 $\mu\text{g}/\text{ml}$ kanamycin and 17.5 $\mu\text{g}/\text{ml}$ chloramphenicol and was subsequently patched onto BG11N+ plates containing Anabaena and RP4. After 24 hours of incubation at 30°C, 30 $\mu\text{g}/\text{ml}$ of neomycin was underlaid; and incubation at 30°C was continued until transconjugants appeared.

25 Individual transconjugants were isolated after conjugation and grown in 2 ml BG11N+ liquid medium with 15 $\mu\text{g}/\text{ml}$ neomycin. Fatty acid methyl esters were prepared from wild type cultures and cultures containing pools of ten transconjugants as follows. Wild type and transgenic cyanobacterial

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1 cultures were harvested by centrifugation and washed
twice with distilled water. Fatty acid methyl esters
were extracted from these cultures as described by
Dahmer *et al.* (1989) *J. Amer. Oil. Chem. Soc.* **66**, 543-
5 548 and were analyzed by Gas Liquid Chromatography
(GLC) using a Tracor-560 equipped with a hydrogen
flame ionization detector and capillary column (30 m x
0.25 mm bonded FSOT Superox II, Alltech Associates
Inc., IL). Retention times and co-chromatography of
10 standards (obtained from Sigma Chemical Co.) were used
for identification of fatty acids. The average fatty
acid composition was determined as the ratio of peak
area of each C18 fatty acid normalized to an internal
standard.

15 Representative GLC profiles are shown in
Fig. 2. C18 fatty acid methyl esters are shown.
Peaks were identified by comparing the elution times
with known standards of fatty acid methyl esters and
were confirmed by gas chromatography-mass
20 spectrometry. Panel A depicts GLC analysis of fatty
acids of wild type Anabaena. The arrow indicates the
migration time of GLA. Panel B is a GLC profile of
fatty acids of transconjugants of Anabaena with
pAM542+1.8F. Two GLA producing pools (of 25 pools
25 representing 250 transconjugants) were identified that
produced GLA. Individual transconjugants of each GLA
positive pool were analyzed for GLA production; two
independent transconjugants, AS13 and AS75, one from
each pool, were identified which expressed significant
30 levels of GLA and which contained cosmids, cSy13 and
cSy75, respectively (Figure 3). The cosmids overlap

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1 in a region approximately 7.5 kb in length. A 3.5 kb
NheI fragment of cSy75 was recloned in the vector
pDUC47 and transferred to Anabaena resulting in gain-
of-function expression of GLA (Table 2).

5 Two NheI/Hind III subfragments (1.8 and 1.7
kb) of the 3.5 kb Nhe I fragment of cSy75-3.5 were
subcloned into "pBLUESCRIPT" (Stratagene) (Figure 3)
for sequencing. Standard molecular biology techniques
were performed as described by Maniatis et al. (1982)
10 and Ausubel et al. (1987). Dideoxy sequencing (Sanger
et al. [1977] Proc. Natl. Acad. Sci. USA 74, 5463-
5467) of pBS1.8 was performed with "SEQUENASE" (United
States Biochemical) on both strands by using specific
oligonucleotide primers synthesized by the Advanced
15 DNA Technologies Laboratory (Biology Department, Texas
A & M University). DNA sequence analysis was done
with the GCG (Madison, WI) software as described by
Devereux et al. (1984) Nucleic Acids Res. 12, 387-395.

Both NheI/HindIII subfragments were
20 transferred into a conjugal expression vector, AM542,
in both forward and reverse orientations with respect
to a cyanobacterial carboxylase promoter and were
introduced into Anabaena by conjugation.
Transconjugants containing the 1.8 kb fragment in the
25 forward orientation (AM542-1.8F) produced significant
quantities of GLA and octadecatetraenoic acid (Figure
2; Table 2). Transconjugants containing other
constructs, either reverse oriented 1.8 kb fragment or
forward and reverse oriented 1.7 kb fragment, did not
30 produce detectable levels of GLA (Table 2).

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1 Figure 2 compares the C18 fatty acid profile
of an extract from wild type Anabaena (Figure 2A) with
that of transgenic Anabaena containing the 1.8 kb
fragment of cSy75-3.5 in the forward orientation
5 (GLC analysis of fatty acid methyl esters
from AM542-1.8F revealed a peak with a retention time
identical to that of authentic GLA standard. Analysis
of this peak by gas chromatography-mass spectrometry
(GC-MS) confirmed that it had the same mass
10 fragmentation pattern as a GLA reference sample.
Transgenic Anabaena with altered levels of
polyunsaturated fatty acids were similar to wild type
in growth rate and morphology.

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1 Table 2 Composition of C18 Fatty Acids in Wild Type
and Transgenic Cyanobacteria

	Strain	Fatty Acid (%)					
		18:0	18:1	18:2	18:3(α)	18:3(γ)	18:4
Wild Type							
	<i>Synechocystis</i> (sp. PCC6803)	13.6	4.5	54.5	-	27.3	-
10	<i>Anabaena</i> (sp. PCC7120)	2.9	24.8	37.1	35.2	-	-
	<i>Synechococcus</i> (sp. PCC7942)	20.6	79.4	-	-	-	-
15	Anabaena Transconjugants						
	cSy75	3.8	24.4	22.3	9.1	27.9	12.5
	cSy75-3.5	4.3	27.6	18.1	3.2	40.4	6.4
20	pAM542 - 1.8P	4.2	13.9	12.1	19.1	25.4	25.4
	pAM542 - 1.8R	7.7	23.1	38.4	30.8	-	-
	pAM542 - 1.7P	2.8	27.8	36.1	33.3	-	-
	pAM542 - 1.7R	2.8	25.4	42.3	29.6	-	-
Synechococcus Transformants							
25	pAM854	27.8	72.2	-	-	-	-
	pAM854 -Δ ¹²	4.0	43.2	46.0	-	-	-
	pAM854 -Δ ⁶	18.2	81.8	-	-	-	-
	pAM854 -Δ ⁶ &Δ ¹²	42.7	25.3	19.5	-	16.5	-

30 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid;
18:3(α), linolenic acid; 18:3(γ), γ-linolenic acid; 18:4,
octadecatetraenoic acid

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EXAMPLE 4**Transformation of Synechococcus
with $\Delta 6$ and $\Delta 12$ Desaturase Genes**

5 A third cosmid, cSy7, which contains a $\Delta 12$ -desaturase gene, was isolated by screening the
10 Synechocystis genomic library with a oligonucleotide synthesized from the published Synechocystis $\Delta 12$ -desaturase gene sequence (Wada *et al.* [1990] Nature (London) 347, 200-203). A 1.7 kb AvaI fragment from
15 this cosmid containing the $\Delta 12$ -desaturase gene was identified and used as a probe to demonstrate that
cSy13 not only contains a $\Delta 6$ -desaturase gene but also
20 a $\Delta 12$ -desaturase gene (Figure 3). Genomic Southern blot analysis further showed that both the $\Delta 6$ -and $\Delta 12$ -desaturase genes are unique in the Synechocystis genome so that both functional genes involved in C18 fatty acid desaturation are linked closely in the
25 Synechocystis genome.

20 The unicellular cyanobacterium Synechococcus (PCC 7942) is deficient in both linoleic acid and GLA(3). The $\Delta 12$ and $\Delta 6$ -desaturase genes were cloned individually and together into pAM854 (Bustos *et al.* [1991] J. Bacteriol. 174, 7525-7533), a shuttle vector that contains sequences necessary for the integration
25 of foreign DNA into the genome of Synechococcus (Golden *et al.* [1987] Methods in Enzymol. 153, 215-231). Synechococcus was transformed with these gene constructs and colonies were selected. Fatty acid methyl esters were extracted from transgenic
30 Synechococcus and analyzed by GLC.

1 Table 2 shows that the principal fatty acids
of wild type Synechococcus are stearic acid (18:0) and
oleic acid (18:1). Synechococcus transformed with
pAM854- Δ 12 expressed linoleic acid (18:2) in addition
5 to the principal fatty acids. Transformants with
pAM854- Δ 6 and Δ 12 produced both linoleate and GLA
(Table 1). These results indicated that Synechococcus
containing both Δ 12- and Δ 6-desaturase genes has
gained the capability of introducing a second double
10 bond at the Δ 12 position and a third double bond at
the Δ 6 position of C18 fatty acids. However, no
changes in fatty acid composition was observed in the
transformant containing pAM854- Δ 6, indicating that in
the absence of substrate synthesized by the Δ 12
15 desaturase, the Δ 6-desaturase is inactive. This
experiment further confirms that the 1.8 kb
NheI/HindIII fragment (Figure 3) contains both coding
and promoter regions of the Synechocystis Δ 6-
desaturase gene. Transgenic Synechococcus with
20 altered levels of polyunsaturated fatty acids were
similar to wild type in growth rate and morphology.

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EXAMPLE 5

Nucleotide Sequence of $\Delta 6$ -Desaturase

The nucleotide sequence of the 1.8 kb
5 fragment of cSy75-3.5 including the functional $\Delta 6$ -
desaturase gene was determined. An open reading frame
encoding a polypeptide of 359 amino acids was
identified (Figure 4). A Kyte-Doolittle hydropathy
analysis (Kyte *et al.* [1982] *J. Mol. Biol.* **157**, 105-
10 132) identified two regions of hydrophobic amino acids
that could represent transmembrane domains (Figure
1A); furthermore, the hydropathic profile of the $\Delta 6$ -
desaturase is similar to that of the $\Delta 12$ -desaturase
gene (Figure 1B; Wada *et al.*) and $\Delta 9$ -desaturases
15 (Thiede *et al.* [1986] *J. Biol. Chem.* **261**, 13230-
13235). However, the sequence similarity between the
Synechocystis $\Delta 6$ - and $\Delta 12$ -desaturases is less than 40%
at the nucleotide level and approximately 18% at the
amino acid level.

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EXAMPLE 6

Transfer of Cyanobacterial Δ^6 -Desaturase into Tobacco

The cyanobacterial Δ^6 -desaturase gene was
5 mobilized into a plant expression vector and transferred to tobacco using Agrobacterium mediated gene transfer techniques. To ensure that the transferred desaturase is appropriately expressed in leaves and developing seeds and that the desaturase
10 gene product is targeted to the endoplasmic reticulum or the chloroplast, various expression cassettes with Synechocystis Δ -desaturase open reading frame (ORF) were constructed. Components of these cassettes include: (i) a 35S promoter or seed specific promoter
15 derived from the sunflower helianthinin gene to drive Δ^6 -desaturase gene expression in all plant tissues or only in developing seeds respectively, (ii) a putative signal peptide either from carrot extensin gene or sunflower helianthinin gene to target newly
20 synthesized Δ^6 -desaturase into the ER, (iii) an ER lumen retention signal sequence (KDEL) at the COOH-terminal of the Δ^6 -desaturase ORF, and (iv) an optimized transit peptide to target Δ^6 desaturase into the chloroplast. The 35S promoter is a derivative of
25 pRTL2 described by Restrepo et al. (1990). The optimized transit peptide sequence is described by Van de Broeck et al. (1985). The carrot extensin signal peptide is described by Chen et al (1985) EMBO J. 9, 2145.
30 Transgenic tobacco plants were produced containing a chimeric cyanobacterial desaturase gene,

1 comprised of the Synechocystis Δ^6 desaturase gene fused
to an endoplasmic reticulum retention sequence (KDEL)
and extensin signal peptide driven by the CaMV 35S
promoter. PCR amplifications of transgenic tobacco
5 genomic DNA indicate that the Δ^6 desaturase gene was
incorporated into the tobacco genome. Fatty acid
methyl esters of leaves of these transgenic tobacco
plants were extracted and analyzed by Gas Liquid
Chromatography (GLC). These transgenic tobacco
10 accumulated significant amounts of GLA (Figure 4).
Figure 4 shows fatty acid methyl esters as determined
by GLC. Peaks were identified by comparing the
elution times with known standards of fatty acid
methyl ester. Accordingly, cyanobacterial genes
15 involved in fatty acid metabolism can be used to
generate transgenic plants with altered fatty acid
compositions.

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EXAMPLE 7

Construction of Borage cDNA library

Membrane bound polysomes were isolated from
5 borage seeds 12 days post pollination (12 DPP) using
the protocol established for peas by Larkins and
Davies (1975 Plant Phys. 55:749-756). RNA was
extracted from the polysomes as described by Mechler
(1987 Methods in Enzymology 152:241-248, Academic
10 Press).

Poly-A+ RNA was isolated from the membrane
bound polysomal RNA by use of Oligotex-dT beads
(Qiagen). Corresponding cDNA was made using
Stratagene's ZAP cDNA synthesis kit. The cDNA library
15 was constructed in the lambda ZAP II vector
(Stratagene) using the lambda ZAP II vector kit. The
primary library was packaged in Gigapack II Gold
packaging extract (Stratagene). The library was used
to generate expressed sequence tags (ESTs), and
20 sequences corresponding to the tags were used to scan
the GenBank database.

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EXAMPLE 8
Hybridization Protocol

Hybridization probes for screening the
5 borage cDNA library were generated by using random
primed DNA synthesis as described by Ausubel *et al*
(1994 Current Protocols in Molecular Biology, Wiley
Interscience, N.Y.) and corresponded to previously
identified abundantly expressed seed storage protein
10 cDNAs. Unincorporated nucleotides were removed by use
of a G-50 spin column (Boehringer Manheim). Probe was
denatured for hybridization by boiling in a water bath
for 5 minutes, then quickly cooled on ice. Filters
for hybridization were prehybridized at 60°C for 2-4
15 hours in prehybridization solution (6XSSC [Maniatis *et*
al 1984 Molecular Cloning A Laboratory Manual, Cold
Spring Harbor Laboratory], 1X Denharts Solution, 0.05%
sodium pyrophosphate, 100 µg/ml denatured salmon sperm
DNA). Denatured probe was added to the hybridization
20 solution (6X SSC, 1X Denharts solution, 0.05% sodium
pyrophosphate, 100 µg/ml denatured salmon sperm DNA)
and incubated at 60°C with agitation overnight.
Filters were washed in 4x, 2x, and 1x SET washes for
15 minutes each at 60°C. A 20X SET stock solution is
25 3M NaCl, 0.4 M Tris base, 20 mM Na₂EDTA-2H₂O. The 4X
SET wash was 4X SET, 12.5 mM PO₄, pH 6.8 and 0.2% SDS.
The 2X SET wash was 2X SET, 12.5 mM PO₄, pH 6.8 and
0.2% SDS. The 1X SET wash was 1X SET, 12.5 mM PO₄, pH
6.8 and 0.2% SDS. Filters were allowed to air dry and
30 were then exposed to X-ray film for 24 hours with
intensifying screens at -80°C.

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EXAMPLE 9

**Random sequencing of cDNAs from a borage seed
(12 DPP) membrane-bound polysomal library**

5 The borage cDNA library was plated at low density (500 pfu on 150 mm petri dishes). Highly prevalent seed storage protein cDNAs were "subtracted" by screening with the previously identified corresponding cDNAs. Non-hybridizing plaques were 10 excised using Stratagene's excision protocol and reagents. Resulting bacterial colonies were used to inoculate liquid cultures and were either sequenced manually or by an ABI automated sequencer. Each cDNA was sequenced once and a sequence tag generated from 15 200-300 base pairs. All sequencing was performed by cycle sequencing (Epicentre). Over 300 ESTs were generated. Each sequence tag was compared to GenBank database by BLASTX computer program and a number of lipid metabolism genes, including the $\Delta 6$ -desaturase 20 were identified.

20 Database searches with a cDNA clone designated mbp-65 using BLASTX with the GenBank database resulted in a significant match to the Synechocystis $\Delta 6$ -desaturase. It was determined however, that this clone was not a full length cDNA. 25 A full length cDNA was isolated using mbp-65 to screen the borage membrane-bound polysomal library. The sequence of the isolated cDNA was determined (Fig. 5A, SEQ ID NO:4) and the protein sequence of the open 30 reading frame (Fig. 5B, SEQ ID NO:5) was compared to other known desaturases using Geneworks

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1 (IntelligGenetics) protein alignment program (Fig. 2).
This alignment indicated that the cDNA was the borage
Δ6-desaturase gene.

5 Although similar to other known plant
desaturases, the borage delta 6-desaturase is distinct
as indicated in the dendrogram shown in Fig. 6.
Furthermore, comparison of the amino acid sequences
characteristic of desaturases, particularly those
proposed to be involved in metal binding (metal box 1
10 and metal box 2), illustrates the differences between
the borage delta 6-desaturase and other plant
desaturases (Table 3).

The borage delta 6-desaturase is
distinguished from the cyanobacterial form not only in
15 over all sequence (Fig. 6) but also in the lipid box,
metal box 1 and metal box 2 amino acid motifs (Table
3). As Table 3 indicates, all three motifs are novel
in sequence. Only the borage delta 6-desaturase metal
box 2 shown some relationship to the Synechocystis
20 delta-6 desaturase metal box 2.

In addition, the borage delta 6-desaturase
is also distinct from another borage desaturase gene,
the delta-12 desaturase. P1-81 is a full length cDNA
that was identified by EST analysis and shows high
25 similarity to the Arabidopsis delta-12 desaturase (Fad
2). A comparison of the lipid box, metal box 1 and
metal box 2 amino acid motifs (Table 3) in borage
delta 6 and delta-12 desaturases indicates that little
homology exists in these regions. The placement of
30 the two sequences in the dendrogram in Fig. 6
indicates how distantly related these two genes are.

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Table 3. Comparison of common amino acid motifs in membrane-bound desaturases

Desaturase	Amino Acid Motif	Lipid Box		Metal Box 1		Metal Box 2	
		SEQ.	ID.	SEQ.	ID.	SEQ.	ID.
Borage Δ^6	WIGHDAGH (SEQ. ID. NO: 6)	HNAHH	(SEQ. ID. NO: 12)	FQIEHH	(SEQ. ID. NO: 20)		
Synechocystis Δ^6	NVGDANH (SEQ. ID. NO: 7)	HNYLHH	(SEQ. ID. NO: 13)	HQVTHH	(SEQ. ID. NO: 21)		
Arab. chloroplast Δ^{15}	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH	(SEQ. ID. NO: 14)	HVIHH	(SEQ. ID. NO: 22)		
Rice Δ^{15}	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH	(SEQ. ID. NO: 14)	HVIHH	(SEQ. ID. NO: 22)		
Glycine chloroplast Δ^{15}	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH	(SEQ. ID. NO: 14)	HVIHH	(SEQ. ID. NO: 22)		
Arab. fad3 (Δ^{15})	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH	(SEQ. ID. NO: 14)	HVIHH	(SEQ. ID. NO: 22)		
Brassica fad3 (Δ^{15})	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH	(SEQ. ID. NO: 14)	HVIHH	(SEQ. ID. NO: 22)		
Borage Δ^2 (P1-81)*	VIAHECGH (SEQ. ID. NO: 9)	HRRHH	(SEQ. ID. NO: 15)	HVIHH	(SEQ. ID. NO: 22)		
Arab. fad2 (Δ^{12})	VIAHECGH (SEQ. ID. NO: 9)	HRRHH	(SEQ. ID. NO: 15)	HVAHH	(SEQ. ID. NO: 23)		
Arab. chloroplast Δ^{12}	VIGHDCAH (SEQ. ID. NO: 10)	HDRHH	(SEQ. ID. NO: 16)	HVIHH	(SEQ. ID. NO: 23)		
Glycine Plastid Δ^{12}	VIGHDCAH (SEQ. ID. NO: 10)	HDRHH	(SEQ. ID. NO: 16)	HVAHH	(SEQ. ID. NO: 23)		
Spinach Plastidial n-6	VIGHDCAH (SEQ. ID. NO: 10)	HDQHH	(SEQ. ID. NO: 17)	HIPHH	(SEQ. ID. NO: 24)		
Synechocystis Δ^{12}	VVGHDGHH (SEQ. ID. NO: 11)	HDHHHH	(SEQ. ID. NO: 18)	HIPHH	(SEQ. ID. NO: 24)		
Anabaena Δ^{12}	VLGHDCGH (SEQ. ID. NO: 8)	HNHHHH	(SEQ. ID. NO: 19)	HVPHH	(SEQ. ID. NO: 25)		

*P1-81 is a full length cDNA which was identified by ESR analysis and shows high similarity to the *Arabidopsis* Δ^{12} desaturase (fad2)

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EXAMPLE 10

Construction of 222.1 Δ^6 NOS for transient and expression

5 The vector pBI221 (Jefferson et al. 1987
EMBO J. 6:3901-3907) was prepared for ligation by
digestion with BamHI and EcoICR I (Promega) which
excises the GUS coding region leaving the 35S promoter
and NOS terminator intact. The borage Δ 6-desaturase
10 cDNA was excised from the Bluescript plasmid
(Stratagene) by digestion with BamHI and XhoI. The
XhoI end was made blunt by use of the Klenow fragment.
This fragment was then cloned into the BamHI/EcoICR I
sites of pBI221, yielding 221. Δ^6 NOS (Fig. 7). In
15 221. Δ^6 .NOS, the remaining portion (backbone) of the
restriction map depicted in Fig. 7 is pBI221.

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EXAMPLE 11

Construction of 121. Δ^6 .NOS for stable transformation

The vector pBI121 (Jefferson et al. 1987
5 EMBO J. 6:3901-3907) was prepared for ligation by
digestion with BamHI and EcoICR I (Promega) which
excises the GUS coding region leaving the 35S promoter
and NOS terminator intact. The borage Δ 6-desaturase
10 cDNA was excised from the Bluescript plasmid
(Stratagene) by digestion with BamHI and XhoI. The
XhoI end was made blunt by use of the Klenow fragment.
This fragment was then cloned into the BamHI/EcoICR I
sites of pBI121, yielding 121.1 Δ^6 NOS (Fig. 7). In
121. Δ^6 .NOS, the remaining portion (backbone) of the
15 restriction map depicted in Fig. 7 is pBI121.

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EXAMPLE 12
Transient Expression

All work involving protoplasts was performed
5 in a sterile hood. One ml of packed carrot suspension
cells were digested in 30 mls plasmolyzing solution
(25 g/l KC1, 3.5 g/l CaCl₂·H₂O, 10mM MES, pH 5.6 and
0.2 M mannitol) with 1% cellulase, 0.1% pectolyase,
and 0.1% dreisalase overnight, in the dark, at room
10 temperature. Released protoplasts were filtered
through a 150 µm mesh and pelleted by centrifugation
(100x g, 5 min.) then washed twice in plasmolyzing
solution. Protoplasts were counted using a double
chambered hemocytometer. DNA was transfected into the
15 protoplasts by PEG treatment as described by Nunberg
and Thomas (1993 Methods in Plant Molecular Biology
and Biotechnology, B.R. Glick and J.E. Thompson, eds.
pp. 241-248) using 10⁶ protoplasts and 50-70 ug of
plasmid DNA (221.Δ6.NOS). Protoplasts were cultured
20 in 5 mls of MS media supplemented with 0.2M mannitol
and 3 µm 2,4-D for 48 hours in the dark with shaking.

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EXAMPLE 13

Stable transformation of tobacco

121.Δ⁶.NOS plasmid construction was used to
5 transform tobacco (*Nicotiana tabacum* cv. *xanthi*) via
Agrobacterium according to standard procedures (Horsch
et al., 1985 *Science* 227: 1229-1231; Bogue et al.,
1990 *Mol. Gen. Genet.* 221:49-57), except that initial
transformants were selected on 100 ug/ml kanamycin.

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SUBSTITUTE SHEET (RULE 26)

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EXAMPLE 14

Preparation and analysis of
fatty acid methyl esters (FAMEs)

Tissue from transfected protoplasts and
5 transformed tobacco plants was frozen in liquid
nitrogen and lyophilized overnight. FAMEs were
prepared as described by Dahmer et al (1989 J. Amer.
Oil Chem. Soc. 66:543-548). In some cases, the
solvent was evaporated again, and the FAMEs were
10 resuspended in ethyl acetate and extracted once with
deionized water to remove any water soluble
contaminants. The FAMEs were analyzed by gas
chromatography (GC) on a J&W Scientific DB-wax column
(30 m length, 0.25 mm ID, 0.25 um film).

15 An example of a transient assay is shown in
Fig. 8 which represents three independent
transfections pooled together. The addition of the
borage $\Delta 6$ -desaturase cDNA corresponds with the
appearance of gamma linolenic acid (GLA) which is one
20 of the possible products of $\Delta 6$ -desaturase.

Figures 9 and 10 depict GC profiles of the
FAMES derived from leaf and seed tissue, respectively,
of control and transformed tobacco plants. Figure 9A
provides the profile of leaf tissue of wild-type
25 tobacco (xanthi); Figure 9B provides the profile of
leaf tissue from a tobacco plant transformed with the
borage $\Delta 6$ desaturase under the transcriptional
control of the 35S CaMV promoter (pBI 121 Δ^6 NOS).
Peaks correspond to 18:2, 18:3 γ (GLA), 18:3 α and 18:4
30 (octadecanonic acid). Figure 10A shows the GC profile
of seeds of a wild-type tobacco; Figure 10B shows the

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1 profile of seed tissue of a tobacco plant transformed
 with pBI 121 Δ^6 NOS. Peaks correspond to 18:2,
 18:3 γ (GLA) and 18:3 α .

5 The relative distribution of the C₁₈ fatty acids in control and transgenic tobacco seeds is shown in Table 4.

TABLE 4

	Fatty Acid	Xanthi	pBI121 Δ^6 NOS
10	18:0	4.0%	2.5%
	18:1	13%	13%
	18:2	82%	82%
	18:3 γ (GLA)	-	2.7%
15	18:3 α	0.82%	1.4%

20 The foregoing results demonstrate that GLA is incorporated into the triacylglycerides of transgenic tobacco leaves and seeds containing the borage Δ^6 -desaturase.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Rhone-Poulenc Agrochimie
- (ii) TITLE OF INVENTION: PRODUCTION OF GAMMA LINOLENIC ACID BY A DELTA 6-DESATURASE

(iii) NUMBER OF SEQUENCES: 25

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: Scully, Scott, Murphy & Presser
- (B) STREET: 400 Garden City Plaza
- (C) CITY: Garden City
- (D) STATE: New York
- (E) COUNTRY: United States
- (F) ZIP: 11530

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE: 30-DEC-1994
- (C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Presser, Leopold
- (B) REGISTRATION NUMBER: 19,827
- (C) REFERENCE/DOCKET NUMBER: 8383ZYXW

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: (516) 742-4343
- (B) TELEFAX: (516) 742-4366
- (C) TELEX: 230 901 SANS UR

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3588 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 2002..3081

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCTAGCCACC	AGTGACGATG	CCTTGAATTG	GGCCATTCTG	ACCCAGGCC	GTATTCTGAA	60
TCCCCGCATT	CGCATTGTTA	ATCGTTGTT	CAACCATGCC	CTGGGTAAAC	GTTCAGACAC	120
CACCTTGCCA	GACCACGTTA	GTTTGAGTGT	TTCCGCCCTG	GCGGCCCCGA	TTTTTCTT	180
TGCGGCTTG	GGCAATCAGG	CGATCGGGCA	ATTGCGTTG	TTTGACCAAGA	CTTGGCCCAT	240
TCAGGAAATT	GTCATTCAAC	AAGACCATCC	CTGGCTCAAT	TTACCCCTGG	CGGATTTATG	300
GGATGATCCG	AGCCGAATGT	TGATCTATTA	CCTACCGGCC	CACAGTGAAA	CGGATTTAGT	360
AGGCGCAGTG	GTGAATAATT	TAACGTTGCA	ATCTGGGAC	CATTTAATAG	TGGGACAAAA	420
ACCCCAACCC	AAGACCAAAC	GGCGATCGCC	TTGGCGAAA	TTTCCAAAC	TGATTACAA	480
CCTGCGGGAG	TATCAGCGGT	ATGTCCAACA	GGTGATATGG	GTGGTGTGTT	TTTTATTGTT	540
GATGATTTTT	CTGGCCACCT	TCATCTACGT	TTCCATTGAT	CAACATATTG	CCCCAGTGGA	600
CGCGTTGTAT	TTTCCGTGG	GCATGATTAC	CGGGGCCGGT	GGCAAGGAAG	AGGTGGCCGA	660
AAAGTCCCCC	GATATCATCA	AAGTATTACAC	AGTGGTGATG	ATGATCGCCG	GGGCGGGGGT	720
GATTGGTATT	TGTTATGCC	TACTGAATGA	TTTCATCCTT	GGCAGTCGCT	TTAGTCAGTT	780
TTTGGATGCG	GCCAAGTTAC	CCGATCGCCA	TCACATCATC	ATTGTGGGC	TGGGGGGAGT	840
GAGCATGGCC	ATTATTGAAG	AGTTAATTCA	CCAGGGCCAT	GAAATTGTGG	TAATCGAAAA	900
GGATACAGAT	AATCGTTCT	TGCATAACGGC	CCGCTCCCTG	GGGGTGCCCC	TAATTGTGGA	960
GGATGCCCGC	CTAGAAAGAA	CGTIGGCCTG	CGCCAATATC	AACCGAGCCG	AAGCCATTGT	1020
GGTGGCCACC	AGCGACGACA	CCGTTAACCTT	GGAAATTGGC	CTAACTGCCA	AGGCGATCGC	1080
CCCTAGCCTG	CCAGTGGTGT	TGCGTTGCCA	GGATGCCAG	TTTAGCCTGT	CCCTGCAGGA	1140
AGTATTGAA	TTGAAACGG	TGCTTTGTCC	GGCGGAATTG	GCCACCTATT	CCTTGCAGGC	1200
GGCGGCCCTG	GGGGGCAAAA	TTTGGGCAA	CGGCATGACC	GATGATTGTC	TGTGGGTAGC	1260
CCTAGCCACC	TTAATCACTC	CTAACCATCC	CTTGCCGAC	CAATTGGTTA	AAATTGCAGC	1320
CCAAAAGTCT	GATTCGTTTC	CCCTCTATCT	AGAACGGGGT	GGCAAAACCA	TCCATAGCTG	1380
GGAATTATTG	GGTACCCATC	TCGACTCTGG	AGACGTGTTG	TATTTAACCA	TGCCCGCCAC	1440
TGCCCTAGAG	CAACTTGGC	GATCGCCCCG	TGCCACTGCT	GATCCTCTGG	ACTTTTTTT	1500

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GGTTTAGCAT GGGGGGATGG AACTCTTGAC TCGGCCAAT GGTGATCAAG AAAGAACGCT	1560
TTGTCTATGT TTAGTATTT TAAGTTAACC AACAGCAGAG GATAACTTCC AAAAGAAATT	1620
AAGCTAAAAA AGTAGCAAAA TAAGTTTAAT TCATAACTGA GTTTTACTGC TAAACAGCGG	1680
TGCAAAAAAG TCAGATAAAA TAAAAGCTTC ACTTCGGTTT TATATTGTGA CCATGGTTCC	1740
CAGGCATCTG CTCTAGGGAG TTTTCCGCT GCCTTAGAG AGTATTTCT CCAAGTCGGC	1800
TAACCTCCCCC ATTTTAAAGGC AAAATCATAT ACAGACTATC CCAATATTGC CAGAGCTTG	1860
ATGACTCACT GTAGAAGGCA GACTAAAATT CTAGCAATGG ACTCCCAGTT GGAATAAATT	1920
TTTATCTCC CCCGGCGCTG GAGTTTTTT GTAGTTAATG GCGGTATAAT GTGAAAGTT	1980
TTTATCTATT TAAATTTATA A ATG CTA ACA GCG GAA AGA ATT AAA TTT ACC Met Leu Thr Ala Glu Arg Ile Lys Phe Thr	2031
1 5 10	
CAG AAA CGG GGG TTT CGT CGG GTA CTA AAC CAA CGG GTG GAT GCC TAC Gln Lys Arg Gly Phe Arg Val Leu Asn Gln Arg Val Asp Ala Tyr	2079
15 20 25	
TTT GCC GAG CAT GGC CTG ACC CAA AGG GAT AAT CCC TCC ATG TAT CTG Phe Ala Glu His Gly Leu Thr Gln Arg Asp Asn Pro Ser Met Tyr Leu	2127
30 35 40	
AAA ACC CTG ATT ATT GTG CTC TGG TTG TTT TCC GCT TGG GCC TTT GTG Lys Thr Leu Ile Ile Val Leu Trp Leu Phe Ser Ala Trp Ala Phe Val	2175
45 50 55	
CTT TTT GCT CCA GTT ATT TTT CCG GTG CGC CTA CTG GGT TGT ATG GTT Leu Phe Ala Pro Val Ile Phe Pro Val Arg Leu Leu Gly Cys Met Val	2223
60 65 70	
TTG GCG ATC GCC TTG GCG GCC TTT TCC TTC AAT GTC GGC CAC GAT GCC Leu Ala Ile Ala Leu Ala Phe Ser Phe Asn Val Gly His Asp Ala	2271
75 80 85 90	
AAC CAC AAT GCC TAT TCC TCC AAT CCC CAC ATC AAC CGG GTT CTG GGC Asn His Asn Ala Tyr Ser Ser Asn Pro His Ile Asn Arg Val Leu Gly	2319
95 100 105	
ATG ACC TAC GAT TTT GTC GGG TTA TCT AGT TTT CTT TGG CGC TAT CGC Met Thr Tyr Asp Phe Val Gly Leu Ser Ser Phe Leu Trp Arg Tyr Arg	2367
110 115 120	
CAC AAC TAT TTG CAC CAC ACC TAC ACC AAT ATT CTT GGC CAT GAC GTG His Asn Tyr Leu His His Thr Tyr Thr Asn Ile Leu Gly His Asp Val	2415
125 130 135	
GAA ATC CAT GGA GAT GGC GCA GTA CGT ATG AGT CCT GAA CAA GAA CAT Glu Ile His Gly Asp Gly Ala Val Arg Met Ser Pro Glu Gln Glu His	2463
140 145 150	

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GTT GGT ATT TAT CGT TTC CAG CAA TTT TAT ATT TGG GGT TTA TAT CTT Val Gly Ile Tyr Arg Phe Gln Gln Phe Tyr Ile Trp Gly Leu Tyr Leu 155 160 165 170	2511
TTC ATT CCC TTT TAT TGG TTT CTC TAC GAT GTC TAC CTA GTG CTT AAT Phe Ile Pro Phe Tyr Trp Phe Leu Tyr Asp Val Tyr Leu Val Leu Asn 175 180 185	2559
AAA GGC AAA TAT CAC GAC CAT AAA ATT CCT CCT TTC CAG CCC CTA GAA Lys Gly Lys Tyr His Asp His Lys Ile Pro Pro Phe Gln Pro Leu Glu 190 195 200	2607
TTA GCT AGT TTG CTA GGG ATT AAG CTA TTA TGG CTC GGC TAC GTT TTC Leu Ala Ser Leu Leu Gly Ile Lys Leu Leu Trp Leu Gly Tyr Val Phe 205 210 215	2655
GGC TTA CCT CTG GCT CTG GGC TTT TCC ATT CCT GAA GTA TTA ATT GGT Gly Leu Pro Leu Ala Leu Gly Phe Ser Ile Pro Glu Val Leu Ile Gly 220 225 230	2703
GCT TCG GTA ACC TAT ATG ACC TAT GGC ATC GTG GTT TGC ACC ATC TTT Ala Ser Val Thr Tyr Met Thr Tyr Gly Ile Val Val Cys Thr Ile Phe 235 240 245 250	2751
ATG CTG GCC CAT GTG TTG GAA TCA ACT GAA TTT CTC ACC CCC GAT GGT Met Leu Ala His Val Leu Glu Ser Thr Glu Phe Leu Thr Pro Asp Gly 255 260 265	2799
GAA TCC GGT GCC ATT GAT GAC GAG TGG GCT ATT TGC CAA ATT CGT ACC Glu Ser Gly Ala Ile Asp Asp Glu Trp Ala Ile Cys Gln Ile Arg Thr 270 275 280	2847
ACG GCC AAT TTT GCC ACC AAT AAT CCC TTT TGG AAC TGG TTT TGT GGC Thr Ala Asn Phe Ala Thr Asn Asn Pro Phe Trp Asn Trp Phe Cys Gly 285 290 295	2895
GGT TTA AAT CAC CAA GTT ACC CAC CAT CTT TTC CCC AAT ATT TGT CAT Gly Leu Asn His Gln Val Thr His His Leu Phe Pro Asn Ile Cys His 300 305 310	2943
ATT CAC TAT CCC CAA TTG GAA AAT ATT ATT AAG GAT GTT TGC CAA GAG Ile His Tyr Pro Gln Leu Glu Asn Ile Ile Lys Asp Val Cys Gln Glu 315 320 325 330	2991
TTT GGT GTG GAA TAT AAA GTT TAT CCC ACC TTC AAA GCG GCG ATC GCC Phe Gly Val Glu Tyr Lys Val Tyr Pro Thr Phe Lys Ala Ala Ile Ala 335 340 345	3039
TCT AAC TAT CGC TGG CTA GAG GCC ATG GGC AAA GCA TCG TGACATTGCC Ser Asn Tyr Arg Trp Leu Glu Ala Met Gly Lys Ala Ser	3088
TTGGGATTGA AGCAAAATGG CAAAATCCCT CGTAAATCTA TGATCGAACG CTTTCTGTTG	3148
CCCGCCGACC AAATCCCCGA TGCTGACCAA AGGTTGATGT TGGCATTGCT CCAAACCCAC	3208

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TTTGAGGGGG	TTCATTGGCC	GCAGTTCAA	GCTGACCTAG	GAGGCAAAGA	TTGGGTGATT	3268
TTGCTCAAAT	CCGCTGGGAT	ATTGAAAGGC	TTCACCACCT	TTGGTTTCTA	CCCTGCTCAA	3328
TGGGAAGGAC	AAACCGTCAG	AATTGTTAT	TCTGGTGACA	CCATCACCGA	CCCATCCATG	3388
TGGTCTAACC	CAGCCCTGGC	CAAGGCTTGG	ACCAAGGCCA	TGCAAATTCT	CCACGAGGCT	3448
AGGCCAGAAA	AATTATATTG	GCTCCTGATT	TCTTCCGGCT	ATCGCACCTA	CCGATTTTG	3508
AGCATTTTTG	CCAAGGAATT	CTATCCCCAC	TATCTCCATC	CCACTCCCCC	GCCTGTACAA	3568
AATTTTATCC	ATCAGCTAGC					3588

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 359 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Leu Thr Ala Glu Arg Ile Lys Phe Thr Gln Lys Arg Gly Phe Arg
 1 5 10 15
 Arg Val Leu Asn Gln Arg Val Asp Ala Tyr Phe Ala Glu His Gly Leu
 20 25 30
 Thr Gln Arg Asp Asn Pro Ser Met Tyr Leu Lys Thr Leu Ile Ile Val
 35 40 45
 Leu Trp Leu Phe Ser Ala Trp Ala Phe Val Leu Phe Ala Pro Val Ile
 50 55 60
 Phe Pro Val Arg Leu Leu Gly Cys Met Val Leu Ala Ile Ala Leu Ala
 65 70 75 80
 Ala Phe Ser Phe Asn Val Gly His Asp Ala Asn His Asn Ala Tyr Ser
 85 90 95
 Ser Asn Pro His Ile Asn Arg Val Leu Gly Met Thr Tyr Asp Phe Val
 100 105 110
 Gly Leu Ser Ser Phe Leu Trp Arg Tyr Arg His Asn Tyr Leu His His
 115 120 125
 Thr Tyr Thr Asn Ile Leu Gly His Asp Val Glu Ile His Gly Asp Gly
 130 135 140
 Ala Val Arg Met Ser Pro Glu Gln Glu His Val Gly Ile Tyr Arg Phe
 145 150 155 160

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Gln Gln Phe Tyr Ile Trp Gly Leu Tyr Leu Phe Ile Pro Phe Tyr Trp
 165 170 175
 Phe Leu Tyr Asp Val Tyr Leu Val Leu Asn Lys Gly Lys Tyr His Asp
 180 185 190
 His Lys Ile Pro Pro Phe Gln Pro Leu Glu Leu Ala Ser Leu Leu Gly
 195 200 205
 Ile Lys Leu Leu Trp Leu Gly Tyr Val Phe Gly Leu Pro Leu Ala Leu
 210 215 220
 Gly Phe Ser Ile Pro Glu Val Leu Ile Gly Ala Ser Val Thr Tyr Met
 225 230 235 240
 Thr Tyr Gly Ile Val Val Cys Thr Ile Phe Met Leu Ala His Val Leu
 245 250 255
 Glu Ser Thr Glu Phe Leu Thr Pro Asp Gly Glu Ser Gly Ala Ile Asp
 260 265 270
 Asp Glu Trp Ala Ile Cys Gln Ile Arg Thr Thr Ala Asn Phe Ala Thr
 275 280 285
 Asn Asn Pro Phe Trp Asn Trp Phe Cys Gly Gly Leu Asn His Gln Val
 290 295 300
 Thr His His Leu Phe Pro Asn Ile Cys His Ile His Tyr Pro Gln Leu
 305 310 315 320
 Glu Asn Ile Ile Lys Asp Val Cys Gln Glu Phe Gly Val Glu Tyr Lys
 325 330 335
 Val Tyr Pro Thr Phe Lys Ala Ala Ile Ala Ser Asn Tyr Arg Trp Leu
 340 345 350
 Glu Ala Met Gly Lys Ala Ser
 355

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1884 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGCTTCACTT CGGTTTTATA TTGTGACCAT GGTTCCCAGG CATCTGCTCT AGGGAGTTTT	60
TCCGCTGCCT TTAGAGAGTA TTTTCTCCAA GTCGGCTAAC TCCCCATTT TTAGGCAAAA	120

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TCATATAACAG ACTATCCAA TATTGCCAGA GCTTTGATGA CTCACTGTAG AAGGCAGACT	180
AAAATTCTAG CAATGGACTC CCAGTTGGAA TAAATTTTA GTCTCCCCG GCGCTGGAGT	240
TTTTTGATAG TTAATGGCGG TATAATGTGA AAGTTTTTA TCTATTTAAA TTTATAAATG	300
CTAACAGCGG AAAGAATTAA ATTTACCCAG AAACGGGGT TTCGTCGGGT ACTAAACCAA	360
CGGGTGGATG CCTACTTTGC CGAGCATGGC CTGACCCAAA GGGATAATCC CTCCATGTAT	420
CTGAAAACCC TGATTATTGT GCTCTGGTTG TTTCCGCTT GGGCCTTGT GCTTTTGCT	480
CCAGTTATTT TTCCGGTGCG CCTACTGGGT TGTATGGTTT TGGCGATCGC CTTGGCGGCC	540
TTTCCTTCA ATGTCGGCCA CGATGCCAAC CACAATGCCT ATTCCCTCAA TCCCCACATC	600
AACC GGTTTC TGGGCATGAC CTACGATTTT GTCGGGTTAT CTAGTTTCT TTGGCGCTAT	660
CGCCACAACT ATTTGCACCA CACCTACACC AATATTCTTG GCCATGACGT GGAAATCCAT	720
GGAGATGGCG CAGTACGTAT GAGTCCTGAA CAAGAACATG TTGGTATTITA TCGTTTCCAG	780
CAATTTATA TTTGGGGTTT ATATCTTTTC ATTCCCTTTT ATTGGTTTCT CTACGATGTC	840
TACCTAGTGC TTAATAAAAGG CAAATATCAC GACCATAAAA TTCCCTCTT CCAGCCCCTA	900
GAATTAGCTA GTTGCTAGG GATTAAGCTA TTATGGCTCG GCTACGTTT CCGCTTACCT	960
CTGGCTCTGG GCTTTCCAT TCCTGAAGTA TTAATTGGTG CTTCGGTAAC CTATATGACC	1020
TATGGCATCG TGGTTTGCAC CATCTTATG CTGGCCCATG TGTTGGAATC AACTGAATT	1080
CTCACCCCCG ATGGTGAATC CGGTGCCATT GATGACGAGT GGGCTATTG CCAAATTCGT	1140
ACCACGGCCA ATTTGCCAC CAATAATCCC TTTTGGAACT GGTTTGTGG CGGTTAAAT	1200
CACCAAGTTA CCCACCACATCT TTTCCCAAT ATTTGTACATA TTCACTATCC CCAATTGGAA	1260
AATATTATTA AGGATGGTTG CCAAGAGTTT GGTGTGGAAT ATAAAGTTA TCCCACCTTC	1320
AAAGCGGCGA TCGCCTCTAA CTATCGCTGG CTAGAGGCCA TGGGCAAAGC ATCGTGACAT	1380
TGCCTTGGGA TTGAAGCAA ATGGCAAAAT CCCTCGTAAA TCTATGATCG AAGCCTTCT	1440
GTTGCCGCC GACCAAATCC CCGATGCTGA CCAAAGGTTG ATGTTGGCAT TGCTCCAAAC	1500
CCACTTTGAG GGGGTTCAATT GGCGCAGTT TCAAGCTGAC CTAGGAGGCA AAGATTGGGT	1560
GATTTTGCTC AAATCCGCTG GGATATTGAA AGGCTTCACC ACCTTTGGTT TCTACCCCTGC	1620
TCAATGGGAA GGACAAACCG TCAGAATTGT TTATTCTGGT GACACCACCA CCGACCCATC	1680
CATGTGGTCT AACCCAGCCC TGGCCAAGGC TTGGACCAAG GCCATGCAAA TTCTCCACGA	1740
GGCTAGGCCA GAAAAATTAT ATTGGCTCCT GATTTCTTCC GGCTATCGCA CCTACCGATT	1800

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TTTGAGCATT TTGCCAAGG AATTCTATCC CCACTATCTC CATCCCACTC CCCGCCTGT 1860
 ACAAAATTTT ATCCATCAGC TAGC 1884

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1685 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AATATCTGCC TACCCTCCCA AAGAGAGTAG TCATTTTCA TCAATGGCTG CTCAAATCAA	60
GAAATACATT ACCTCAGATG AACTCAAGAA CCACGATAAA CCCGGAGATC TATGGATCTC	120
GATTCAAGGG AAAGCCTATG ATGTTTCGGA TTGGGTGAAA GACCATCCAG GTGGCAGCTT	180
TCCCTTGAAG AGTCTTGCTG GTCAAGAGGT AACTGATGCA TTTGTTGCAT TCCATCCTGC	240
CTCTACATGG AAGAATCTTG ATAAGTTTT CACTGGGTAT TATCTTAAAG ATTACTCTGT	300
TTCTGAGGTT TCTAAAGATT ATAGGAAGCT TGTGTTGAG TTTTCTAAAA TGGGTTTGT	360
TGACAAAAAA GGTCAATATTA TGTTTGCAAC TTTGTGCTTT ATAGCAATGC TGTTTGCTAT	420
GAGTGTGTTAT GGGGTTTGT TTTGTGAGGG TGTTTGTTA CATTGTTTT CTGGGTGTTT	480
GATGGGGTTT CTTTGGATTC AGAGTGGTTG GATTGGACAT GATGCTGGC ATTATATGGT	540
AGTGTCTGAT TCAAGGCTTA ATAAGTTTAT GGGTATTGTT GCTGCAAATT GTCTTTCAGG	600
AATAAGTATT GGTTGGTGG AATGGAACCA TAATGCACAT CACATTGCCT GTAATAGCCT	660
TGAATATGAC CCTGATTTAC AATATATACC ATTCTTGTT GTGTCTTCCA AGTTTTTGG	720
TTCACTCACC TCTCATTCT ATGAGAAAAG GTTGACTTTT GACTCTTTAT CAAGATTCTT	780
TGTAAGTTAT CAACATTGGA CATTTCACCC TATTATGTGT GCTGCTAGGC TCAATATGTA	840
TGTACAATCT CTCATAATGT TGTTGACCAA GAGAAATGTG TCCTATCGAG CTCAGGAAC	900
CTTGGGATGC CTAGTGTCT CGATTGGTA CCCGTTGCTT GTTTCTTGT TGCCTAATTG	960
GGGTGAAAGA ATTATGTTG TTATTGCAAG TTTATCAGTG ACTGGAATGC AACAAAGTTCA	1020
GTTCTCCTTG AACCACCTCT CTTCAAGTGT TTATGTTGGA AAGCCTAAAG GGAATAATTG	1080
GTGAGAAA CAAACGGATG GGACACTTGA CATTCTTGT CCTCCTTGGA TGGATTGGTT	1140
TCATGGTGGGA TTGCAATTCC AAATTGAGCA TCATTTGTTT CCCAAGATGC CTAGATGCAA	1200

CCTTAGGAAA	ATCTCGCCCT	ACGTGATCGA	GTTATGCAAG	AAACATAATT	TGCCTTACAA	1260
TTATGCATCT	TTCTCCAAGG	CCAATGAAAT	GACACTCAGA	ACATTGAGGA	ACACAGCATT	1320
GCAGGGCTAGG	GATATAACCA	AGCCGCTCCC	GAAGAATTG	GTATGGGAAG	CTCTTCACAC	1380
TCATGGTTAA	AATTACCCCT	AGTTCATGTA	ATAATTGAG	ATTATGTATC	TCCTATGTTT	1440
GTGTCTTGTC	TTGGTTCTAC	TTGTTGGAGT	CATTGCAACT	TGTCTTTAT	GGTTTATTAG	1500
ATGTTTTTA	ATATATTTA	GAGGTTTGC	TTTCATCTCC	ATTATTGATG	AATAAGGAGT	1560
TGCATATTGT	CAATTGTTGT	GCTCAATATC	TGATATTTG	GAATGTACTT	TGTACCACTG	1620
TGTTTCAGT	TGAAGCTCAT	GTGTACTTCT	ATAGACTTTG	TTTAAATGGT	TATGTCATGT	1680
TATTT						1685

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 448 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met	Ala	Ala	Gln	Ile	Lys	Lys	Tyr	Ile	Thr	Ser	Asp	Glu	Leu	Lys	Asn
1					5				10					15	
His	Asp	Lys	Pro	Gly	Asp	Leu	Trp	Ile	Ser	Ile	Gln	Gly	Lys	Ala	Tyr
					20				25				30		
Asp	Val	Ser	Asp	Trp	Val	Lys	Asp	His	Pro	Gly	Gly	Ser	Phe	Pro	Leu
					35				40				45		
Lys	Ser	Leu	Ala	Gly	Gln	Glu	Val	Thr	Asp	Ala	Phe	Val	Ala	Phe	His
					50.				55			60			
Pro	Ala	Ser	Thr	Trp	Lys	Asn	Leu	Asp	Lys	Phe	Phe	Thr	Gly	Tyr	Tyr
					65				70			75		80	
Leu	Lys	Asp	Tyr	Ser	Val	Ser	Glu	Val	Ser	Lys	Asp	Tyr	Arg	Lys	Leu
					85				90				95		
Val	Phe	Glu	Phe	Ser	Lys	Met	Gly	Leu	Tyr	Asp	Lys	Lys	Gly	His	Ile
					100				105				110		
Met	Phe	Ala	Thr	Leu	Cys	Phe	Ile	Ala	Met	Leu	Phe	Ala	Met	Ser	Val
					115				120				125		
Tyr	Gly	Val	Leu	Phe	Cys	Glu	Gly	Val	Leu	Val	His	Leu	Phe	Ser	Gly
					130				135				140		

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Cys Leu Met Gly Phe Leu Trp Ile Gln Ser Gly Trp Ile Gly His Asp
145 150 155 160

Ala Gly His Tyr Met Val Val Ser Asp Ser Arg Leu Asn Lys Phe Met
165 170 175

Gly Ile Phe Ala Ala Asn Cys Leu Ser Gly Ile Ser Ile Gly Trp Trp
180 185 190

Lys Trp Asn His Asn Ala His His Ile Ala Cys Asn Ser Leu Glu Tyr
195 200 205

Asp Pro Asp Leu Gln Tyr Ile Pro Phe Leu Val Val Ser Ser Lys Phe
210 215 220

Phe Gly Ser Leu Thr Ser His Phe Tyr Glu Lys Arg Leu Thr Phe Asp
225 230 235 240

Ser Leu Ser Arg Phe Phe Val Ser Tyr Gln His Trp Thr Phe Tyr Pro
245 250 255

Ile Met Cys Ala Ala Arg Leu Asn Met Tyr Val Gln Ser Leu Ile Met
260 265 270

Leu Leu Thr Lys Arg Asn Val Ser Tyr Arg Ala Gln Glu Leu Leu Gly
275 280 285

Cys Leu Val Phe Ser Ile Trp Tyr Pro Leu Leu Val Ser Cys Leu Pro
290 295 300

Asn Trp Gly Glu Arg Ile Met Phe Val Ile Ala Ser Leu Ser Val Thr
305 310 315 320

Gly Met Gln Gln Val Gln Phe Ser Leu Asn His Phe Ser Ser Ser Val
325 330 335

Tyr Val Gly Lys Pro Lys Gly Asn Asn Trp Phe Glu Lys Gln Thr Asp
340 345 350

Gly Thr Leu Asp Ile Ser Cys Pro Pro Trp Met Asp Trp Phe His Gly
355 360 365

Gly Ser Gln Phe Gln Ile Glu His His Leu Phe Pro Lys Met Pro Arg
370 375 380

Cys Asn Leu Arg Lys Ile Ser Pro Tyr Val Ile Glu Leu Cys Lys Lys
385 390 395 400

His Asn Leu Pro Tyr Asn Tyr Ala Ser Phe Ser Lys Ala Asn Glu Met
405 410 415

Thr Leu Arg Thr Leu Arg Asn Thr Ala Leu Gln Ala Arg Asp Ile Thr
420 425 430

Lys Pro Leu Pro Lys Asn Leu Val Trp Glu Ala Leu His Thr His Gly
435 440 445

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Trp Ile Gly His Asp Ala Gly His
1 5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asn Val Gly His Asp Ala Asn His
1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Val Leu Gly His Asp Cys Gly His
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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Val Ile Ala His Glu Cys Gly His
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Val Ile Gly His Asp Cys Ala His
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Val Val Gly His Asp Cys Gly His
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

His Asn Ala His His
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

His Asn Tyr Leu His His
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

His Arg Thr His His
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

His Arg Arg His His
1 5

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(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

His Asp Arg His His
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

His Asp Gln His His
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

His Asp His His His
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

His Asn His His His
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Phe Gln Ile Glu His His
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

His Gln Val Thr His His
1 5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

His Val Ile His His
1 5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

His Val Ala His His
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

His Ile Pro His His
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

His Val Pro His His
1 5

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1 WHAT IS CLAIMED:

1. An isolated nucleic acid encoding a borage
Δ6-desaturase.

5

2. The isolated nucleic acid of Claim 1
comprising the nucleotide sequence of SEQ ID NO: 4.

10 3. An isolated nucleic acid that codes for the
amino acid sequence of SEQ ID NO: 5.

4. A vector comprising the nucleic acid of any
one Claims 1-3.

15

5. An expression vector comprising the
isolated nucleic acid of any one of Claims 1-3 operably
linked to a promoter and optionally a termination signal
capable of effecting expression of the gene product of
said isolated nucleic acid.

20

6. The expression vector of Claim 5 wherein
said promoter is a Δ-6 desaturase promoter, an Anabaena
carboxylase promoter, a helianthinin promoter, a glycinin
promoter, a napin promoter, the 35S promoter from CaMV, or
25 a helianthinin tissue-specific promoter.

7. The expression vector of Claim 5 wherein
said promoter is constitutive or tissue-specific.

30

8. The expression vector of Claim 5 wherein
said termination signal is a Synechocystis termination

35

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1 signal, a nopaline synthase termination signal, or a seed
termination signal.

9. A cell comprising the vector of any one of
5 Claims 4-8.

10. The cell of Claim 9 wherein said cell is an
animal cell, a bacterial cell, a plant cell or a fungal
cell.

10

11. A transgenic organism comprising the
isolated nucleic acid of any one of Claims 1-3.

15

12. A transgenic organism comprising the vector
of any one of Claims 4-8.

13. The transgenic organism of Claim 11 or 12
wherein said organism is a bacterium, a fungus, a plant or
an animal.

20

14. A plant or progeny of said plant which has
been regenerated from the plant cell of Claim 10.

25

15. The plant of Claim 14 wherein said plant is
a sunflower, soybean, maize, tobacco, peanut, carrot or
oil seed rape plant.

30

16. A method of producing a plant with
increased gamma linolenic acid (GLA) content which
comprises:

35

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1 (a) transforming a plant cell with the isolated
nucleic acid of any one of Claims 1-3; and
 (b) regenerating a plant with increased GLA
content from said plant cell.

5

17. A method of producing a plant with
increased gamma linolenic acid (GLA) content which
comprises:

10 (a) transforming a plant cell with the vector of
any one of Claims 4-8; and
 (b) regenerating a plant with increased GLA
content from said plant cell.

15 18. The method of Claim 16 or 17 wherein said
plant is a sunflower, soybean, maize, tobacco, peanut,
carrot or oil seed rape plant.

20 19. A method of inducing production of gamma
linolenic acid (GLA) in an organism deficient or lacking
in GLA which comprises transforming said organism with the
isolated nucleic acid of any one of Claims 1-3.

25 20. A method of inducing production of gamma
linolenic acid (GLA) in an organism deficient or lacking
in GLA which comprises transforming said organism with the
vector of any one of Claims 4-8.

30 21. A method of inducing production of gamma
linolenic acid (GLA) in an organism deficient or lacking
in GLA and linoleic acid (LA) which comprises transforming
said organism with an isolated nucleic acid encoding

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1 borage $\Delta 6$ -desaturase and an isolated nucleic acid encoding
 $\Delta 12$ -desaturase.

22. The method of Claim 21 wherein said
5 isolated nucleic acid encoding $\Delta 6$ -desaturase comprises
nucleotides 44 to 1390 of SEQ. ID NO: 4.

23. A method of inducing production of
octadecatetraenoic acid in an organism deficient or
10 lacking in gamma linolenic acid which comprises
transforming said organism with the isolated nucleic acid
of any one of Claims 1-3.

24. A method of inducing production of
15 octadecatetraenoic acid in an organism deficient or
lacking in gamma linolenic acid which comprises
transforming said organism with the vector of any one of
Claims 4-8.

20 25. The method of Claim 23 or 24 wherein said
organism is a bacterium, a fungus, a plant or an animal.

26. A method of producing a plant with improved
chilling resistance which comprises:

25 (a) transforming a plant cell with the isolated
nucleic acid of any one of Claims 1-3; and
(b) regenerating said plant with improved
chilling resistance from said transformed plant cell.

30 27. A method of producing a plant with improved
chilling resistance which comprises:

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1 (a) transforming a plant cell with the vector of
any one of Claims 4-8; and

 (b) regenerating said plant with improved
chilling resistance from said transformed plant cell.

5

28. The method of Claim 26 or 27 wherein said
plant is a sunflower, soybean, maize, tobacco, peanut,
carrot or oil seed rape plant.

10

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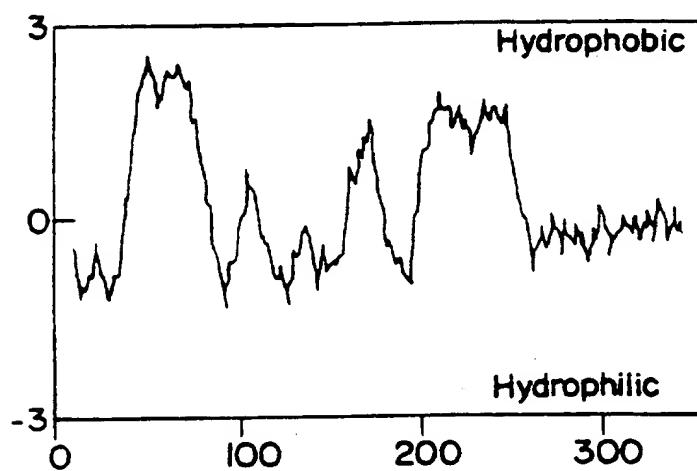


FIG. IA

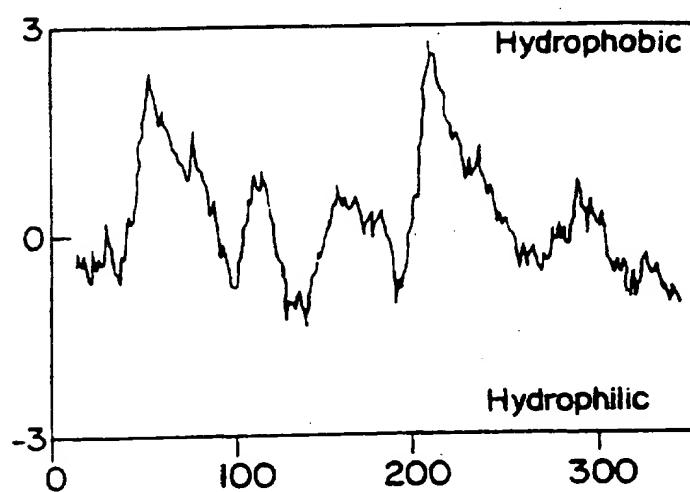
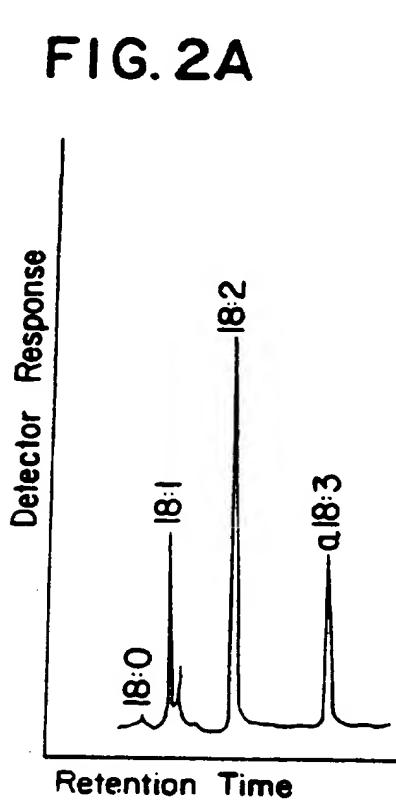
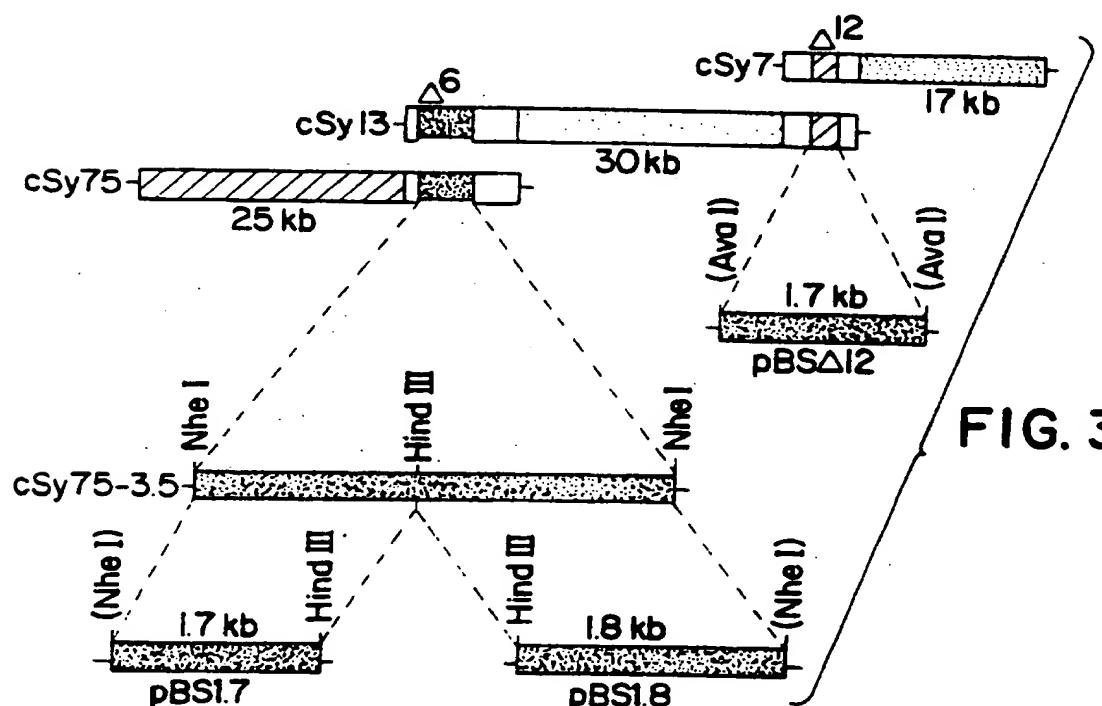
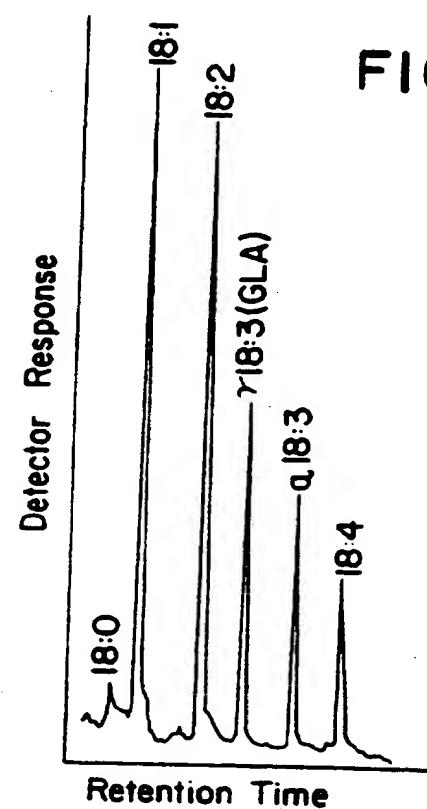


FIG. IB

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FIG. 2A**FIG. 2B****FIG. 3**

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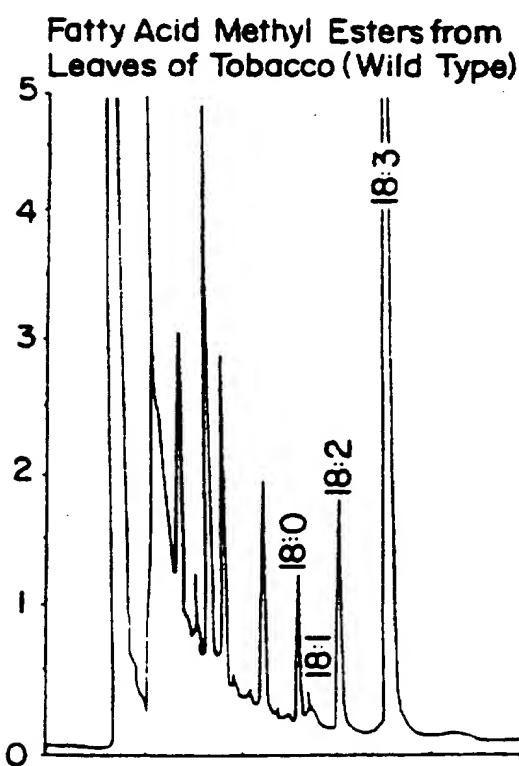


FIG. 4A

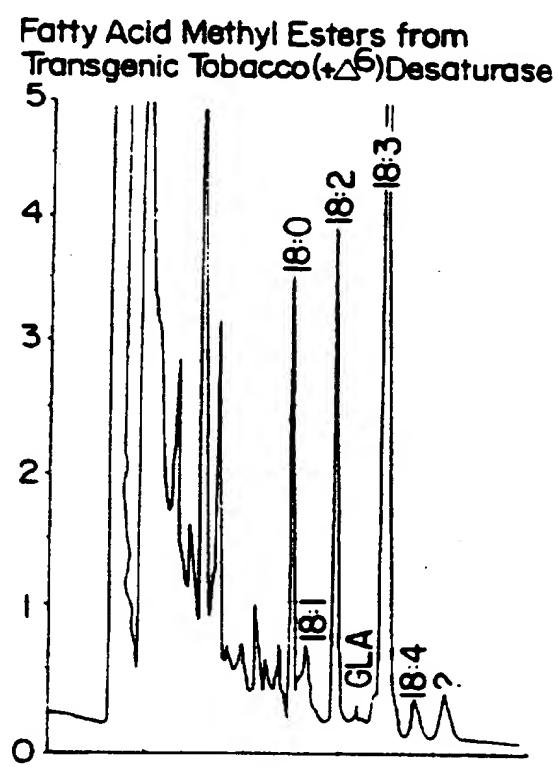


FIG. 4B

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FIG. 5A

1 aatatctgcc tacccctccca aaggaggtgg tcaattttca
 81 aactcaagaa ccacgataaa cccggagatc tcccttgaag agtcttgctg
 161 gaccatcccg gtggcagctt ttggatggaa ataaatctgg
 241 ctctacatgg aagaatctgg attaaatctgg
 321 attaggaatctt tggtttttagg ttttctaaaa
 401 attagcaatcc ttgttgtcat gaggtgtttat gggttttgg
 481 gatggdtttt ctggatgttc aaggatgtttc gattggacat
 561 ataaggattttt gggtttttt gttggaaattt tttttttttt
 801 catttaccc tattatgtgt tttttttttt
 881 tcctatcgag cttagggactt ttatgtcaag
 961 gggtggaaaga attatgtttt tttttttttt
 721 ttcaatccacc tctcatctt attagatgtt tttttttttt
 801 cttcaatgtt ttatgtggaa aaggcttaaa
 881 cttccatggaa tttttttttt tttttttttt
 961 cttatgtcc tttttttttt tttttttttt
 1041 cttcaatgtt ttatgtggaa aaggcttaaa
 1121 cttccatggaa tttttttttt tttttttttt
 1201 cttatgtcc tttttttttt tttttttttt
 1281 ccaatggaaat gacactcggaa acattggaa acacggatc
 1361 gtatggaaag ctcttccacac tttttttttt tttttttttt
 1441 gtgtttttttt tttttttttt tttttttttt
 1521 gaggtttttttt tttttttttt tttttttttt
 1601 gatgtttttttt tttttttttt tttttttttt
 1681 taatgtt
 1 tttttttttt
 801 aatccatgg tttttttttt tttttttttt
 161 gttttttttt tttttttttt tttttttttt
 241 ttgtttttttt tttttttttt tttttttttt
 321 ttgtttttttt tttttttttt tttttttttt
 401 ttgtttttttt tttttttttt tttttttttt
 481 ttgtttttttt tttttttttt tttttttttt
 561 ttgtttttttt tttttttttt tttttttttt
 801 ttgtttttttt tttttttttt tttttttttt
 881 ttgtttttttt tttttttttt tttttttttt
 961 ttgtttttttt tttttttttt tttttttttt
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 1281 ttgtttttttt tttttttttt tttttttttt
 1361 ttgtttttttt tttttttttt tttttttttt
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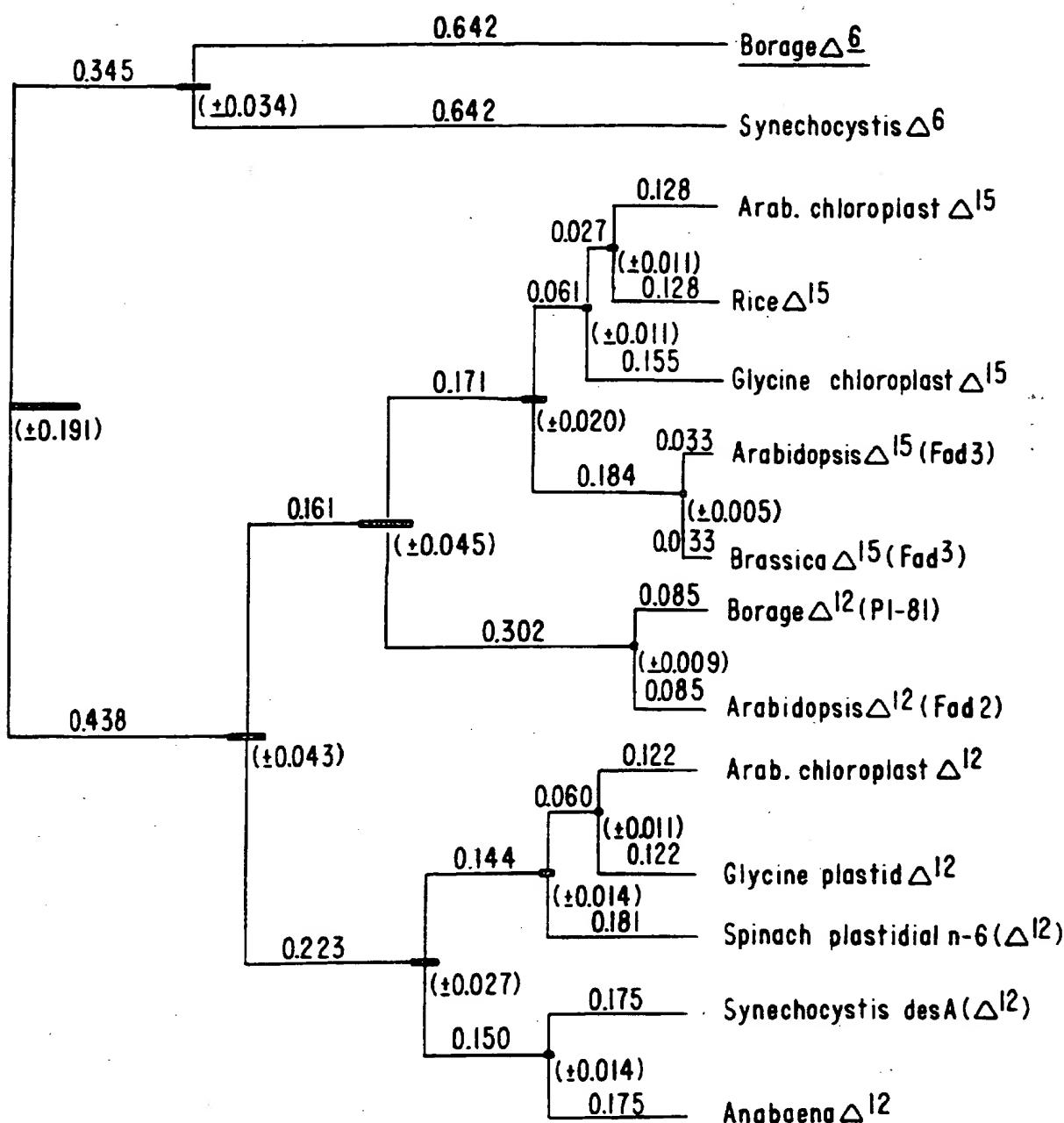
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FIG. 5B

1 MAAQIKKYIT SDELKNHDKP GDLWISIQGK AYDVSDDWVKD HPGGSFPLKS LAGQEVTDAF VAFHPASTWK NLDKFFTGYY 80
81 LKDYSVSEVS KDYRKLVFEF SKMGLYDKKG HIMFATLCFI AMLFAMSVYG VIFCEGVLVH LFSGCLMGFL WIQSGWIGHD 160
161 AGHMVVSDS RLNKFMGIFA ANCLSGISIG WWKWNHNAH IACNSLEYDP DLQYIPFLVV SSKFFGSLTS HFYEKRLTFD 240
241 SLSREFFSYQ HWTFYPIMCA ARLNMYVQSL IMLLTKRNVS YRAQELLGCL VFSTIWYPLLV SCLPNNGERI MFVIASLSVT 320
321 GMQQVQFSLN HFSSSVYVGK PKGNMWF EKO TDGTLDISCP PWDWFWHGGL QOIEHHLF P KMPRCNLRKI SPYVIECKK 400
401 HNLPYNYASF SKANEMTLRT LRNTALQARD ITKPLPKNLV WEALTHNG 448

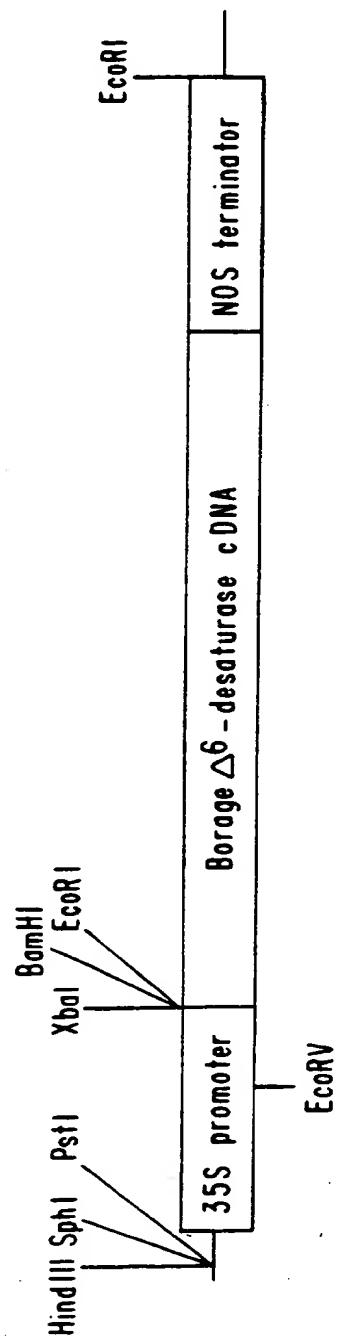
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FIG. 6



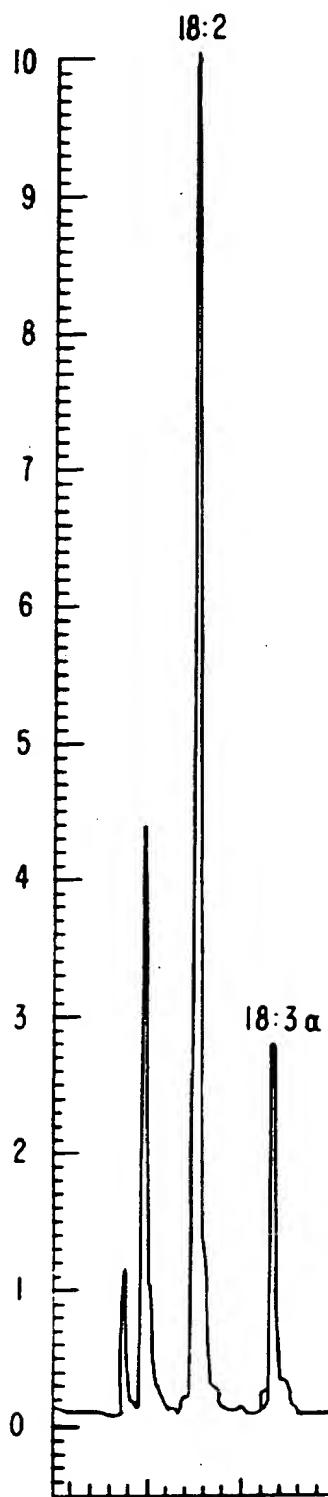
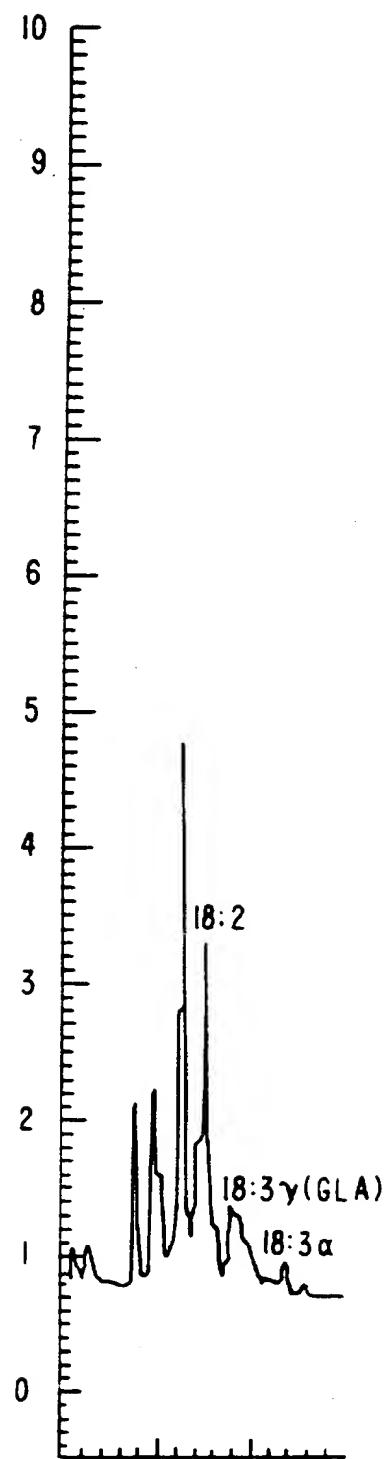
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FIG. 7

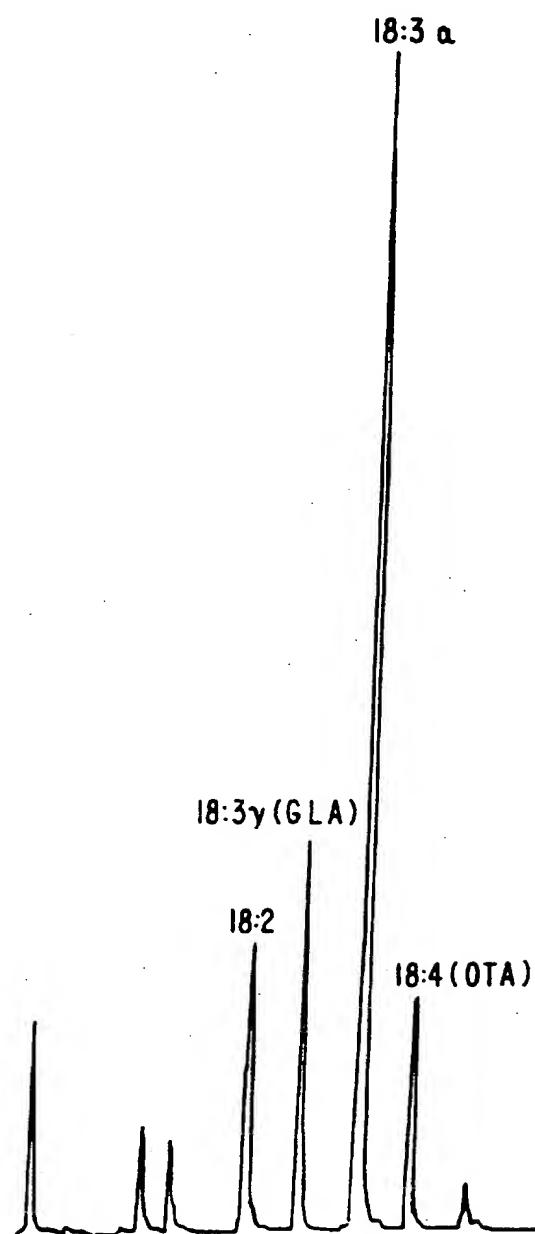
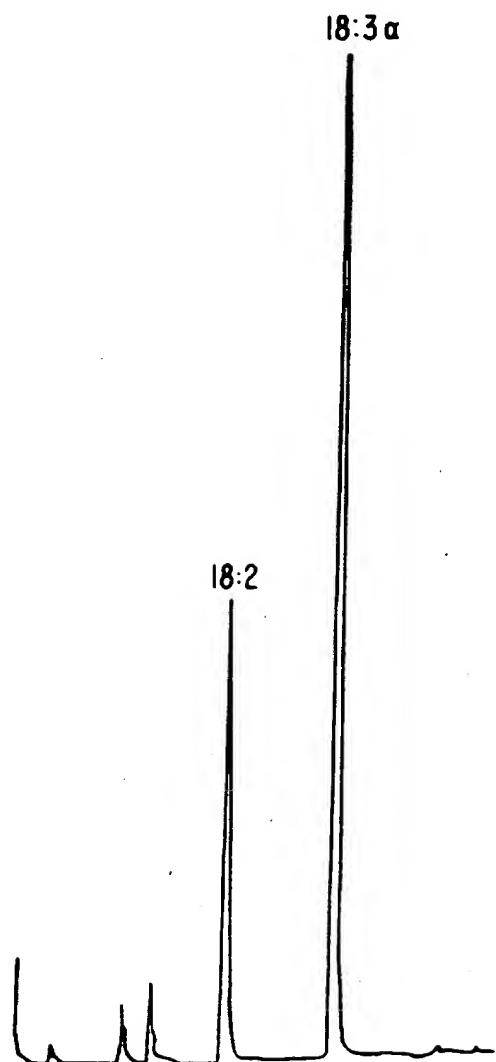


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FIG. 8A**FIG. 8B****SUBSTITUTE SHEET (RULE 26)**

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FIG. 9B**FIG. 9A**

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FIG. 10A

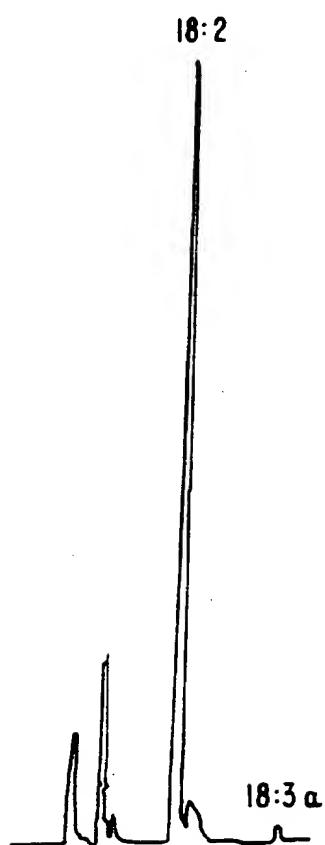
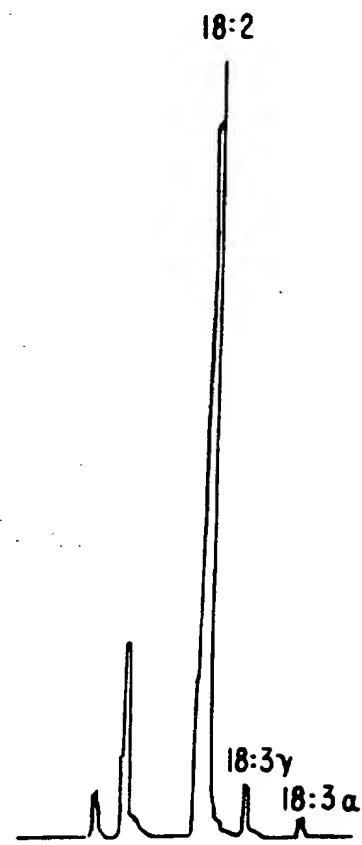


FIG. 10B



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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ :	A3	(11) International Publication Number: WO 96/21022 (43) International Publication Date: 11 July 1996 (11.07.96)
(21) International Application Number:	PCT/IB95/01167	(81) Designated States: AU, BR, CA, CN, JP, RO, RU, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
(22) International Filing Date:	28 December 1995 (28.12.95)	
(30) Priority Data:	08/366,779 30 December 1994 (30.12.94) US	Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(71) Applicant:	RHONE-POULENC AGROCHIMIE [FR/FR]; 14-20, rue Pierre-Baizet, F-69263 Lyon (FR).	(88) Date of publication of the international search report: 12 September 1996 (12.09.96)
(72) Inventors:	THOMAS, Terry, L.; 3004 Normand, College Station, TX 77845 (US). REDDY, Avutu, S.; 3902 E. 29th Street #G11, Bryan, TX 77802 (US). NUCCIO, Michael; P.O. Box 553, College Station, TX 77841 (US). NUNBERG, Andrew, N.; 2804 B. Sprucewood Street, Bryan, TX 77801 (US). FREYSSINET, Georges, L.; 21, rue de Nerville, F-69450 Saint-Cyr-au-Mont-d'Or (FR).	
(74) Agent:	MITSCHERLICH & PARTNER; Sonnenstrasse 33, D-80331 München (DE).	

(54) Title: PRODUCTION OF GAMMA LINOLENIC ACID BY A Δ 6-DESATURASE

(57) Abstract

Linoleic acid is converted into γ -linolenic acid by the enzyme Δ 6-desaturase. The present invention is directed to isolated nucleic acids comprising the Δ 6-desaturase gene. More particularly, the isolated nucleic acid comprises the promoter, coding region and termination regions of the Δ 6-desaturase gene. The present invention provides recombinant constructions comprising the Δ 6-desaturase coding region in functional combination with heterologous regulatory sequences. The nucleic acids and recombinant constructions of the instant invention are useful in the production of GLA in transgenic organisms.

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INTERNATIONAL SEARCH REPORT

International Application No
PL/IB 95/01167

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/53 C12N15/82 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>KADER, J.-C. AND P. MAZLIAK (ED.). PLANT LIPID METABOLISM; 11TH INTERNATIONAL MEETING ON PLANT LIPIDS, PARIS, FRANCE, JUNE 26-JULY 1, 1994. XX+588P. KLUWER ACADEMIC PUBLISHERS: DORDRECHT, NETHERLANDS; NORWELL, MASSACHUSETTS, USA. 0 (0). 1995. 509-511. ISBN: 0-7923-3250-4, XP000569979</p> <p>GALLE A-M, ET AL.: "Solubilization of DELTA-12- and DELTA-6-desaturases from seeds of borage microsomes." see the whole document</p> <p>---</p> <p>-/-</p>	1-28

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Patent family members are listed in annex.

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Date of the actual completion of the international search

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Maddox, A

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 95/01167

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